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THE SECONDARY NODULES OF LYMPH NODES

THEIR RELATION TO CHRONIC INFLAMMATORY PROCESSES *

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The question of the significance of the secondary nodules of lymphatic tissue, which was considered settled by Flemming's [1] work (1885), was again opened by Hellman [2] in 1919. Since that time many workers have contributed to a solution of the problem. The discussion has concerned several apparently opposed facts. Flemming's contention that the secondary nodules were physiologically the chief sites of the regeneration of lymphocytes was supported by the single finding that there were more mitotic figures in the secondary nodules of the lymph nodes examined than in the tissues surrounding the nodules.

Although no serious objection to Flemming's theory was raised in the literature until Hellman's work appeared, there were disclosed in the interim several facts that could not be explained with entire satisfaction by that theory. The absence of germinal centers in fetal life was established. It was recognized that germinal centers are not present in the involved tissues in lymphosarcoma and lymphatic leukemia. Careful cytologic studies revealed that mitotic figures were as abundant in the lymph cords and interfollicular portions as in the secondary nodules, and it was recognized that Flemming's "tingible Körper" were disintegrating cells.

Hellman's proposition that the secondary nodules are the anatomic evidence of work performed by the lymph node in response to lymph-borne or blood-borne foreign material has received the support of many investigators. Other workers have reported their findings in support of Flemming's theory, but none has offered an explanation of the absence of secondary nodules in the fetus and in the lymphatic neoplasias.

Recently many writers, taking the middle ground, have expressed the belief that the secondary nodules represent both the "reaction centers" of Hellman and the "germ centers" of Flemming. There is in the literature considerable confusion, owing on the one hand to a lack of uniform terminology and on the other to a failure on the part of

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1. Flemming, W.: Arch. f. mikr. Anat. **24**:50, 1885.
2. Hellman, T. J.: Upsala läkaref. förh. **24**:57, 217 and 283, 1919.

some authors to appreciate the marked differences between fresh animal material and human postmortem material.

An investigation of the problem was undertaken on the suggestion of Dr. H. E. Robertson, who has long believed that Flemming's theory is inadequate. During the course of this investigation it became apparent that certain types of chronic inflammation are frequently associated with a hyperplasia of the regional lymph nodes. It was thought that some light might be thrown on the question of the nature of the secondary nodules if the constancy of the relationship of the lesions and the lymphoid hyperplasia was determined.

REVIEW OF THE LITERATURE

The secondary nodules of lymphatic tissue were first mentioned by Brücke,[3] who in 1850 noted cloudy-white central spots (zentralen Flecke) in the peripheral nodules of some lymph nodes and more frequently in the nodules of Peyer's patches. Brücke[4] noted that the afferent lymphatics of mesenteric lymph nodes of dogs fed on a diet low in fat contained clear fluid, and that the efferent lymph vessels of the same nodes contained cloudy fluid. He determined that the cloudiness of the lymph was due to the presence of lymphocytes and postulated that the lymphocytes were formed in the lymph nodes. In the medullary cords and cortical nodules, he noted the "various stages of lymphocyte formation," and he therefore designated these portions of the lymph node "Keimlager."

His,[5] in his classic work on lymph nodes, described clear, round areas in the cortical nodules of lymph nodes of the ox and in the nodules of Peyer's patches of the rabbit. He called these clear areas "vacuoles" and noted that their blood supply was capillary only.

Hansen[6] supported the observations of His, and added that there were many nodes in which no "vacuoles" could be found, and that the "vacuoles" were demonstrable only after birth. He considered the light central areas as due to an atrophy brought about by a proliferation of the lymphocytes and a consequent interference with the blood supply. He found no microscopic evidence to account for the increased number of lymphocytes in the efferent lymph vessels.

Frey[7] merely stated that within the large cortical follicles of the lymph nodes of oxen are other clear follicles, which His had called

3. Brücke, Ernst: Ueber den Bau und die physiologische Bedeutung der Peyer'schen Drüsen, Wissenschaftliche Denkschriften der wiener Akademie, 1850, vol. 2, p. 21.

4. Brücke, Ernst: Wien. Akad. Mar. Nat. Kl. **6**:99, 1854.

5. His, W.: Ztschr. f. wissensch. **11**:65 and 416, 1861.

6. Hansen, G. A.: Virchows Arch. f. path. Anat. **56**:280, 1872.

7. Frey, H.: Handbuch der Histologie und Histochemie des Menschen, ed. 4, Leipzig, W. Engelmann, 1874, p. 421.

"vacuoles." Toldt [8] also gave these clear central areas scant mention, stating that they were seen in "some lymph follicles." Toldt used the term lymph node in preference to that of lymph gland.

Flemming [1] established an anatomic and physiologic interpretation of the cortical nodules of lymph nodes that was to remain unchallenged in the literature for many years. He reviewed the literature critically, deploring the continued use of the term lymph gland. He supported the use of the term node instead of the term gland, and the use of the term nodule instead of the term follicle, pointing out that the term follicle was not applicable to a solid structure. He adopted Brücke's designation of the combined medullary cords and cortical nodules as "Keimlager." Flemming considered the "zentralen Flecke" of Brücke and the "Vacuolen" of His as identical with the structures that he called anatomically "secondary nodules" and physiologically "germinal centers" (Keimcentren). Flemming examined three lymph nodes from two oxen, the pancreas asellii and Peyer's patches from two rabbits and the lingual tonsil of a man. He found mitotic figures in the medullary cords and in the lymph sinuses of the lymph nodes, but most plentifully in the light centers of the cortical nodules and Peyer's patches. It was because of the abundance of mitotic figures in these nodules that he called them "germinal centers." He defined the germinal center as a light area with a sharply defined border of compressed reticulum packed with lymphocytes, which in turn was surrounded by an area of less densely packed lymphocytes. The center he described as consisting of cells with abundant cytoplasm and therefore with nuclei widely separated. Among these cells, he noted few lymphocytes and many large cells containing deeply staining bodies, which he designated "tingible Körper." Because some of the mitotic figures were apparently in reticular cells, he considered the possibility that the reticulum might be the "mother substance" from which the lymphocytes were formed. In the lingual tonsil, he found some nodules without mitotic figures, and he explained this on the basis that the specimen was fixed one hour post mortem. He expressed the belief that cells in the process of mitosis might complete their division after death, but that new mitoses would not be initiated. He recognized that the secondary nodules varied in size and concluded that they were labile structures, increasing and decreasing in activity periodically. To explain the scarcity of lymphocytes in the secondary nodule and their accumulation about its periphery, he postulated a centrifugal pressure mechanism that forced the daughter cells to the periphery of the nodule. Flemming had previously noted secondary nodules in spleens, and he concluded unequivocally that the secondary nodules of the lymph nodes,

8. Toldt: Lehrbuch der Gewebelehre, ed. 2, Stuttgart, F. Enke, 1884.

spleen and alimentary tract were the principal places for the formation of lymphocytes. He believed that the fluctuation of the size of the centers was dependent on physiologic stimuli.

Several of Flemming's students carried on studies on other lymphatic structures. Drews studied tonsils of various animals; Moibus and Heilbrunn, spleens of animals and man. These workers supported the findings of Flemming, and Paulsen,[9] another of Flemming's students, studied "hyperplastic" lymphatic structures to determine if the regeneration of lymphocytes was the same in these as in normal lymphatic tissue. Paulsen used a lymph node, a "so-called rheumatic bubo," from a 48 year old man, the "hypertrophic" pharyngeal tonsils of an 18 year old youth and several "hypertrophic" faucial tonsils from persons from 8 to 18 years of age. He described an increase in the number and size of the secondary nodules and an increase in the number of mitotic figures in these nodules. He concluded that regeneration of lymphocytes followed the same plan in these structures as in the normal. He also found the "tingible Körper" to be most abundant in tonsils and postulated a relationship between their number and the number of mitotic figures.

Baumgarten [10] and Ribbert [11] had demonstrated the abundance, and Downey and Weidenreich [12] the preponderance, of mitotic figures outside the secondary nodules; Bunting [13] had expressed the belief that the secondary nodules were the sites of regeneration of lymphatic tissue as a whole; Naegeli [14] had found large "germ centers" in many infectious diseases coincidentally with a lymphopenia and expressed the belief that this was inconsistent with the "germ centers" being a site for production of lymphocytes; Marchand [15] showed some skepticism regarding Flemming's theory, but it remained for Hellman [16] to summarize the accumulated evidence and offer observations of his own in support of a theory opposed to that of Flemming.

Hellman's observations [17] that "germinal centers" are not found in normal fetuses has abundant support in the literature. Gulland [18] examined fetuses of man, rabbit, sheep and guinea-pig and found no

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9. Paulsen, E.: Arch. f. mikr. Anat. **24**:345, 1885.
10. Baumgarten, P.: Ztschr. f. klin. Med. **9**:93, 1885; **10**:24, 1886.
11. Ribbert: Beitr. z. path. Anat. u. z. allg. Path. **6**:187, 1889.
12. Downey, Hal; and Weidenreich, F.: Arch. f. mikr. Anat. **80**:306, 1912.
13. Bunting, T. L.: J. Anat. & Physiol. **39**:55 and 178, 1905.
14. Naegeli, O.: Blutkrankheiten und Blutdiagnostie, Berlin, W. de Gruyter & Company, 1912, p. 279.
15. Marchand, F.: Verhandl. d. deutsch. path. Gesellsch. **16**:5, 1913.
16. Hellman, T. J.: Upsala läkaref. förh. **24**:283, 1919; Beitr. z. path. Anat. u. z. allg. Path. **68**:333, 1921.
17. Hellman (footnote 2, second reference; footnote 16, second reference).
18. Gulland, G. L.: J. Path. & Bact. **2**:447, 1894.

"germinal centers." Baum [19] reported that he and his students had not been able to find "germinal centers" in horse, pig or dog before the fifth day of life nor in the cow before the eleventh day. Gundobin [20] found, on investigating lymph nodes of 50 new-born infants and 100 children from 1 to 2 years of age, that the first "germinal centers" appeared at the age of 2 months, and that typical, well formed "germinal centers" appeared at 2 years of age. Jolly and Rosello [21] found that the spleen of rats did not contain "germinal centers" up to 3 weeks of age, and that the centers did not reach full development until 2 months of age. Goslar,[22] on investigating 34 human tonsils from stillborn infants, living infants and adults, concluded that "germinal centers appear relatively late in extra-uterine life." Parodi,[23] in a study of the lymph nodes of 66 human beings varying from 35 cm. fetuses to adults of 85 years found no "germinal centers" in the fetuses and rarely in persons more than 30 years of age. Ehrich [24] studied chiefly the axillary lymph nodes of 48 fetuses and new-born infants, varying from 14 to 40 weeks in utero, and found "secondary nodules" in a single case only. This was in the spleen of a 34 cm. embryo the mother of which had died of pneumonia. Hellman [25] was unable to find secondary nodules in the tonsils of rabbits before birth, and he was able to find in the literature [26] but a single report of an instance of "germinal centers" in a fetus. This was a case reported by Margerstedt [27] in which there were "germinal centers" in an inflamed appendix. Hellman reported a case of his own in which there were well formed "germinal centers" in the spleen of a 1 day old syphilitic infant. Collins [28] found no secondary nodules in an examination of 50 fetal appendixes.

It appears to be well established, that, except when definite abnormality can be demonstrated, lymphoid tissue in the fetus and the newborn infant is without secondary nodules. But Bunting [29] stated that

19. Baum, H., in Ellenberger, W.: Handbuch der vergleichenden mikroscopischer Anatomie der Haustiere, Berlin, Wilhelm Paul Parey, 1906, vol. 2, p. 105.

20. Gundobin, N. P.: Jahrb. f. Kinderh. **64**:529, 1906.

21. Jolly, J., and Rosello, H.: Compt. rend. Soc. de biol. **66**:40, 1909.

22. Goslar, A.: Beitr. z. path. Anat. u. z. allg. Path. **56**:405, 1913.

23. Parodi, U.: Haematologica **8**:1, 1927.

24. Ehrich, W.: Am. J. Anat. **43**:385, 1929.

25. Hellman (footnote 2, first reference).

26. Hellman (footnote 16, second reference).

27. Margerstedt: Untersuchungen zur normalen und pathologischen Anatomie des Wurmfortstatzes, Diss., Berlin, 1908, cited by Hellman.

28. Collins, D. C.: Personal communication to the author; data to be published.

29. Bunting, C. H.: Diseases of the Lymph Glands, Nelson's Loose Leaf Living Medicine, New York, T. Nelson & Sons, 1921, vol. 3, p. 347.

Sabin found "germinal centers" in embryos. Sabin,[30] in describing the 13 cm. stage of a pig embryo, said, "The second point in advance is the formation of the germinal center. Within the follicle . . . there are small clumps of cells, *definitely lymphocytes,* heaped around a capillary tuft. In the entire node at this stage there are eight of these 'germinal centers'." The structure is obviously not a "germinal center" as described by Flemming. Nor is it a germinal center in the sense in which the term is used generally in the literature. Ehrich [31] pointed out that if the term germinal center is to be used, it should be limited in its application to the structure originally described by Flemming.

Hellman [26] pointed out that secondary nodules are found in many pathologic conditions. He [25] had found, in the course of his studies of the lymph nodes in carcinoma and tuberculosis, that lymphatic hyperplasia was frequent in chronic infections and toxic conditions, and that the secondary nodules played a considerable part in the hyperplasia. Saltzman [32] found an increase in the size and number of "germ-centers" in the gastric mucosa in carcinoma of the stomach. Matko [33] found the "germ-centers" increased in size and number following subcutaneous and intravenous injections of typhoid vaccine. The change was most marked in the regional nodes following subcutaneous injections. Stschastnyi [34] produced similar changes by the injection of proteins. Many workers (Bizzozero,[35] Welch and Flexner,[36] Oertel,[37] Barbacci [38] and others) demonstrated the necrotic type of secondary nodule in diphtheria, and Barbacci produced the same changes in lymph nodes of guinea-pigs by subcutaneous injection of diphtheria toxin.

Hellman [26] further offered in support of his working hypothesis (that the secondary nodules are "reaction centers") his observations concerning the lack of correlation between the blood lymphocyte count and the state of the secondary nodules in rabbits. In 1914, he had found by weight two peaks in the developmental curve of the lymphatic tissue of "normal rabbits." [25] One peak, that at puberty, corresponded with the period at which the rabbit normally has the greatest number

30. Sabin, F. R.: Am. J. Anat. **4**:355, 1905.
31. Ehrich, W.: Am. J. Anat. **43**:347, 1929.
32. Saltzman: Arb. a. d. path. Inst. zu Helsingfors **1**:335, 1913.
33. Matko, J.: Ztschr. f. exper. Path. u. Therap. **19**:437, 1918.
34. Stschastnyi, S. M.: Beitr. z. path. Anat. u. z. allg. Path. **38**:456, 1905.
35. Bizzozero, Giulio: Medezinisches Jahrbuch, 1876, p. 203.
36. Welch, W. H., and Flexner, Simon: Bull. Johns Hopkins Hosp., **1-2**:107, 1891.
37. Oertel, M. J.: Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung, Leipzig, F. C. W. Vogel, 1887; cited by Hellman.
38. Barbacci, O.: Zentralbl. f. allg. Path. u. path. Anat. **7**:321, 1896.

of lymphocytes in the blood.[39] This increase in weight of the lymphatic tissue was due to a hyperplasia of the lymphatic tissue in general, whereas the peak at the age of 10 months was due to an increase only in the peripheral lymphatic tissue. In another work,[25] he had found that rabbits' tonsils reached their greatest development at the age of 10 months, and that their hypertrophy was due to a hyperplasia of the secondary nodules. He argued that the peripheral location of the secondary nodules in the afferent lymph path indicated their significant relationship to incoming material, and that the location of the most highly developed nodules immediately under the epithelium of the respiratory and alimentary tracts argued for the same conclusion.

Hellman laid considerable stress on the sharp demarcation between the secondary nodule and the narrow lymphocyte border, arguing that the persistence of the sharp demarcation and the border in the presence of necrosis of the center (as in diphtheria) indicated that the structure could have nothing to do with the production of lymphocytes. He further stressed his finding that the absolute weight of the dark borders of the nodules (as determined in rabbits' tonsils[40]) always remained the same, regardless of the size of the secondary nodule.

The necessity for distinguishing various types of secondary nodules structurally and functionally was pointed out by Groll and Krampf.[41] They described 6 types of secondary nodule: the "germ center" of Flemming, the epithelioid type first described by Stilling,[42] the hemorrhagic type, the necrotic type (diphtheria), the solid type (consisting of lymphocytes only) and the hyaline type. Of the 300 spleens that they studied, 36 were from "normal" persons. In these 36 spleens 4 types of center were found: the "germ center" and the epithelioid, the hyaline and the solid types up to 25 years of age; thereafter, only the epithelioid and the hyaline types. They concluded that the epithelioid and hyaline types of center represent involutionary changes and are evidence of decreased production of lymphocytes in the spleen. The authors concluded from the examination of the spleens from human beings dying of infectious diseases, pyemia, tuberculosis, cachexia, carcinoma and sarcoma that lymphocyte building in the spleen decreases with age and in these conditions by a failure of the "germ centers." They found no secondary nodules of the active type in the cases of tuberculosis, cachexia, carcinoma or sarcoma. In but 10 per cent of the cases of infections, sepsis and pyemia were there secondary nodules of the active type, whereas 66.66 per cent of the centers in the

39. Lindberg, G.: Folia haemat. **9**:64, 1910.

40. Hellman (footnote 2, second reference).

41. Groll, H., and Krampf, F.: Centralbl. f. allg. Path. u. path. Anat. **31**:145, 1920.

42. Stilling, H.: Virchows Arch. f. path. Anat. **103**:15, 1886.

"normal" cases were of the active type. Groll and Krampf concluded that the necrotic changes appearing in "germ centers," such as are seen particularly in diphtheria, are brought about by toxins.

Heiberg [43] developed the thesis that phagocytosis is the one important faculty of the "functional centers" of lymphatic tissue, and that these centers have nothing to do with the production of lymphocytes. He based his conclusions on his findings in human tonsils (1923, 1924) and other lymphatic tissues (1928). He considered the secondary nodules as the principal place for the destruction of lymphocytes, and the so-called "germ center cells" he believed were probably reserve phagocytes. The basis for such conclusions was: the parallel increase and decrease of the numbers of mitotic figures and phagocytes filled with débris of lymphocytes; the constant presence of disintegrating lymphocytes in the active type of center, and the absence of disintegrating lymphocytes in the quiescent type of center. Heiberg considered the lymphocyte border of the active type of secondary nodule as a lymphocytic infiltration, such as occurs about a degenerative process.

Wätjen [44] concluded from the results obtained by injecting arsenic intravenously into rabbits, dogs and cats that the secondary nodules were both reaction centers and "germ centers." He found karyorrhexis of the cells of the centers only, and because surrounding cells were not fragmented, he considered that the cell fragments in the center were of "lymphoblastic" origin. He believed that the reaction to arsenic was general, rather than specific for arsenic, and he considered the changes in the centers to be of the same type as those described for diphtheria and those reported by Heineke [45] after experimental use of roentgen rays. In many species of animals exposed to roentgen rays, Heineke had found a rapid appearance of nuclear fragments in the secondary nodules of all the lymphatic tissues. This nuclear fragmentation was followed by the appearance of many mitotic figures and marked phagocytosis. These changes in the secondary nodules were found also by Warthin,[46] Jolly and Rosello [21] and others. Heiberg [47] cited these changes as supporting his contention that the secondary nodules are phagocytic structures primarily.

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43. Heiberg, K. A.: Virchows Arch. f. path. Anat. **240**:301, 1923; Acta otolaryng. **6**:190, 1924; **7**:3, 1924; Acta med. Scandinav. **65**:443, 1927; Acta path. et microbiol. Scandinav. (supp.) **5**:43, 1928.

44. Wätjen, J.: Verhandl. d. deutsch. path. Gesellsch. **20**:366, 1925; Virchows Arch. f. Path. Anat. **256**:86, 1925; **271**:556, 1929.

45. Heineke, H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **14**:21, 1905.

46. Warthin, A. S.: Internat. Clin. **4**:243, 1906.

47. Heiberg (footnote 43, first reference).

Heilman [48] interpreted his findings in human appendixes in favor of Hellman's hypothesis. Heilman summarized his views [49] by stating that the "secondary follicles" are formed of hyperplastic reticuloendothelial cells, which are phagocytic for damaged lymphocytes, and act as antibody-forming organs. He considered the "germ center" type of secondary nodule pathologic because it appears after birth.

Parodi,[22] on the basis of a study of 66 spleens of human beings, from 35 cm. fetuses to persons 85 years of age, pointed out that the "lymphoblastic" or "Flemming type" of secondary nodule was rare in such material. He therefore concluded that the lymphoblastic type of secondary nodule was exceedingly sensitive to a state of illness. He further found, as had Downey and Weidenreich [12] and Mollier,[50] that lymphocytes are formed in lymphoid tissue in which no "germ centers" are found. He believed Hellman's hypothesis correct only in that it indicated that the lymph nodule is subject to stimuli from outside the node.

Catania,[51] working in Parodi's laboratory, considered that Hellman's views must be supported on the basis of the appearance of the secondary nodules in Waldeyer's ring and appendix in 33 persons dead of disease.

Rotter [52] examined 200 lymph nodes from 35 human beings and defined 6 types of secondary nodule: (1) the solid, consisting of mature lymphocytes; (2) the epithelioid; (3) the reticular; (4) the lymphoblastic; (5) the necrotic, and (6) the hyaline. He expressed the belief that the secondary nodules arise as a reaction to blood-borne poisons, and that the type of reaction depends on the degree of sensitization of the nodule as brought about by lymphogenous, enterogenous, splenogenous or hematogenous poisons.

Latta [53] concluded, on the basis of human autopsy material, that the light centers of the lymph nodules of the spleen were the result of a degenerative process. He considered the degeneration due to interference with the blood supply of the center of the nodule by the proliferation of the lymphocytes about its border. Latta found that the light centers contained no capillaries, and that they contained more reticular fibers than the surrounding tissue. No mitotic figures were seen in the centers, and little phagocytosis was noted. He had previ-

48. Heilman, P.: Virchows Arch. f. path. Anat. **258**:52, 1925.
49. Heilman, P.: Virchows Arch. f. path. Anat. **259**:160, 1926.
50. Mollier, S.: Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. in München. **29**:14, 1913.
51. Catania, V.: Haematologica **8**:221, 1927.
52. Rotter, W.: Virchows Arch. f. path. Anat. **265**:596, 1927.
53. Latta, J. S.: Anat. Rec. **24**:233, 1923.

ously [54] expressed the belief that the light centers of the lymphatic nodules of the rabbits' intestine were due to interference with the blood supply of the center by proliferation of the cells of the nodule. Such an interpretation of secondary nodules is conceivable on the basis of observations at autopsy in human spleens, in which the commonest types of secondary nodules are the reticular, epithelioid and hyaline. It is not applicable to the "germ center" type of secondary nodule, which contains abundant capillaries, mitotic figures throughout, abundant active phagocytes and fewer reticular fibers than the surrounding tissue.

Uchino,[55] working in Aschoff's laboratory, experimented extensively with subcutaneous and intramuscular injections of nutrose, mixtures of nutrose and bacteria and bacteria alone. He found fairly uniform changes in the lymphatic nodules of the lymph nodes and spleen after repeated injections. Their centers were increased in size and contained more fragmented nuclei and more mitotic figures. The degree of change was proportional to the number of injections. The medullary cords and the internodular cortical tissue took no appreciable part in the reaction. Uchino offered his results in support of Wätjen's [56] contention that both Flemming's and Hellman's views were in part correct. Aschoff [57] expressed a similar view.

Maximow's extensive observations of connective tissue [58] and tissue cultures,[59] led him to support Flemming's theory. Maximow [60] summarized his ideas of the secondary nodules of lymph nodes in Cowdry's Special Cytology. He reported there the results of a cytologic study of the "germ centers" of the lymph nodes of the cat. He described various-sized secondary nodules with varying cellular content. He interpreted these variations as cyclic changes from a resting stage to one of active production of lymphocytes and macrophages. The active phase was followed by stages of regression to the resting stage. In discussing the functional significance of the "germ centers," Maximow stated, "It is quite probable that the lymphoid tissue in general and the germ centers in particular may take part in the defense reactions of the organism. It is also certain that the germ centers are not abso-

54. Latta, J. S.: Am. J. Anat. **24**:159, 1921.

55. Uchino, S.: Beitr. z. path. Anat. u. z. allg. Path. **74**:405, 1925.

56. Wätjen (footnote 44, first reference).

57. Aschoff, L.: Lectures on Pathology: Reticulo-Endothelial System, New York, Paul B. Hoeber, 1924, p. 1.

58. Maximow, A.: Development of the Blood, in von Möllendorff, Wilhelm: Handbuch der Gewebelehre der Menschen, Berlin, Julius Springer, 1929, vol. 2, pt. 1, p. 232.

59. Maximow, A.: Arch. f. mikr. Anat. **97**:283 and 314, 1923.

60. Maximow, A.: Lymphocyte and Plasma Cell, in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, 1928, vol. 1, p. 321.

lutely necessary for the regeneration of lymphocytes, as in the embryo and in the new-born infant, they are not clearly developed." He concluded his discussion with the following quite inconsistent statement: "In view of the described regular cyclic growth phenomena in the germ centers their importance as the *principal* places of regeneration of lymphocytes, as formulated by Flemming, has to be maintained."

That the inconsistence may be due to a misconception regarding the frequency of the occurrence of the secondary nodules of the Flemming type is indicated by a statement made on page 332: "They (germ centers) may disappear and arise again according to the changing *physiological* conditions. . . . As a rule all germ centers of a lymph node, perhaps of the whole body, show similar conditions at a given time." It is well known that secondary nodules of the active type are common in laboratory animals. An explanation is seen in the work of Manfredi [61] and in that of Uchino.[54] Manfredi cultured bacteria from lymph nodes of 88.6 per cent of 88 "normal" animals. Uchino produced proliferative changes in lymphatic nodules by subcutaneous injection of bacteria. These observations, coupled with those concerning the scarcity of secondary nodules of the active type at times of life when man is least exposed to infections, would indicate that their presence is due to pathologic stimuli. There is abundant evidence in the literature (Uchino,[55] Catania,[51] Ehrich,[62] Parodi [23]), and my own observations indicate, that the conditions of the lymph nodules may vary according to the location in the body and even the location in the node.

Lang [63] investigated lymph nodes of rabbits in which myeloid metaplasia had been produced experimentally. He concluded that the secondary nodules of lymphatic tissue contained 2 undifferentiated mesenchymal types of cell, the lymphoblast and the reticular cell, each differentiating according to its stimulus. He considered both Flemming's and Hellman's theories correct in part.

Ehrich reviewed the anatomy [31] and embryology [24] of human lymph nodes and described large nodules in the parenchyma of the lymph nodes of rabbits and human beings, which he designated pseudosecondary nodules. He described these pseudosecondary nodules as a transitional stage between secondary nodules of the Flemming type and "lymphoid tissue" (Aschoff) and between secondary nodules of the solid type and "lymphoid tissue." He considered the pseudosecondary nodule to be the chief site of lymphocyte production in the embryo, in which they first appear about the twenty-second week, at the time the solid secondary nodules appear.

61. Manfredi, L.: Virchows Arch. f. path. Anat. **155**:335, 1899.
62. Ehrich, W.: J. Exper. Med. **49**:361, 1929.
63. Lang, F. J.: Folia haemat. **36**:31, 1928.

Ehrich,[64] using rabbits, produced, by subcutaneous injection of small amounts of cultures of a staphylococcus of very low virulence, a hyperplasia of a local lymph node and a lymphocytosis. The lymphocytosis appeared coincidentally with the regression of the secondary nodules of the Flemming type in the regional lymph node. The secondary nodules of the Flemming type became prominent and were increased in number later, after the lymphocytosis had disappeared. This increase in the number of the secondary nodules appeared to be associated with abscess formation at the site of injection. After injecting killed cultures of staphylococci intravenously into rabbits, Ehrich[62] found a peak in the leukocytosis and lymphocytosis on the tenth day paralleled by an increase in the weight of the peripheral lymph nodes and in that of the spleen. He found a great increase in the number of Flemming's nodules in the spleen and some increase in the size of the nodules. The increase in the number of Flemming's nodules in the peripheral lymph nodes was not so marked. Ehrich's conclusions[65] were that Flemming's theory could not be supported in its original form and questionably in a modified form.

Schillaci[66] studied the behavior of lymph nodes of guinea-pigs in acute and prolonged inanition. He found that the lymphocyte mantles of the secondary nodules decreased as starvation was prolonged, and that, when the animals were fed again, these lymphocyte mantles returned coincidentally with an increase in the number of mitotic figures in the secondary nodule. He concluded that these occurrences could be explained best by Flemming's theory that the centers have a lymphocyte-forming function.

In apparent contradiction to the foregoing interpretation are the observations of Gottesman and Gottesman.[67] They studied autoplastic lymph node transplants in albino rats and noted the parallel development of reticulum and lymphocytes with transitional stages between. The lymphatic nodules were formed late in the regeneration, and only in the latest stages studied (eighth day) was there beginning formation of "germinal centers."

MATERIALS AND METHODS

General studies of the structure of the secondary nodules of lymphatic tissue were made on approximately 1,500 lymph nodes removed from 500 cadavers within four hours after death. The ages of the deceased varied from 6 months in utero to 88 years. Nodes were removed from all the regions in many cases; in others, only nodes from a visceral and a peripheral group were taken. Approximately

64. Ehrich, W.: J. Exper. Med. **49**:347, 1929.
65. Ehrich, W.: Beitr. z. path. Anat. u. z. allg. Path. **86**:285, 1931.
66. Schillaci, G.: Haematologica **9**:397, 1928.
67. Gottesman, J. M., and Gottesman, J.: Proc. Soc. Exper. Biol. & Med. **25**:484, 1928.

200 of the nodes were removed from the pyloric region of the stomach. Spleens in all the cases and submucous lymphatic tissue in many of the cases were studied. The lymph nodes and spleens of 30 rabbits and of several cats, dogs, guinea-pigs and sheep were examined. The human material, as a routine, was fixed in modified Orth's solution, embedded in paraffin and stained with hematoxylin and eosin. The animal material was fixed in 4 per cent formaldehyde. For cytologic studies, Hitchcock and Ehrich's [68] modification of the Pappenheim stain was used on material fixed in Zenker's solution. The Perdrau [69] silver method was used for reticulum.

In addition to the aforementioned materials, lymph nodes, tonsils and appendixes removed at operation were studied in fresh, frozen sections stained with Terry's polychrome methylene blue and in formaldehyde-fixed, frozen sections stained with hematoxylin and eosin.

A special study was made of the lymph nodes resected with a part of the stomach for gastric carcinoma or for gastric ulcer. There were 116 specimens from partial gastrectomies; 98 of these were from gastrectomies for gastric carcinoma and 18 from those for gastric ulcer. The lymph nodes from 43 of the cases of carcinoma had previously been sectioned for a study of metastasis by MacCarthy and Blackford.[70] My studies of the lymph nodes in these 43 cases were made on the fixed, frozen sections stained with hematoxylin and eosin that had been prepared in 1912. The remainder of the nodes from the 116 specimens were taken from specimens that had been preserved in formaldehyde for periods varying from two weeks to seven years. Many nodes were sectioned serially. Sections of the gastric lesion were studied in all cases. The total number of nodes studied from the 116 specimens was 593. Only those nodes not completely replaced by carcinoma were used. In the entire group of persons from whom the specimens came, there were 108 men and 8 women; the average age was 56, the ages ranging from 26 to 76 years.

RECORDED OBSERVATIONS ON RELATIONSHIP BETWEEN GASTRIC LESIONS AND HYPERPLASIA OF REGIONAL LYMPH NODES

Investigations of the relationship between gastric lesions and regional lymph nodes have been concerned chiefly with metastatic involvement of the nodes. Cunèo,[71] however, noted that "hyperplastic glands" were sometimes present in the region of gastric carcinoma without carcinomatous involvement of the nodes. Carle and Fantino [72] had made the same observation. Lengemann [73] mentioned large nodes in the region of a benign ulcer of the stomach and large noncarcinomatous nodes in the region of a gastric carcinoma. MacCarty and Blackford [70] made similar observations. None of these workers commented on secondary nodules, and I have been able to find in the

68. Hitchcock, C. H., and Ehrich, W.: Arch. Path. **9**:625, 1930.

69. Perdrau, J. R.: J. Path. & Bact. **24**:117, 1921.

70. MacCarty, W. C., and Blackford, J. N.: Ann. Surg. **55**:811, 1912.

71. Cunèo, B.: De l'en vahissment du système lymphatique dans le cancer de l'estomac, Thèse de Paris, 1900.

72. Carle, A., and Fantino, G.: Arch. f. klin. Chir. **56**:217, 1898.

73. Lengemann: Arch. f. klin. Chir. **68**:382, 1902.

literature no report concerning the frequency of the occurrence of secondary nodules of the active type in the lymph nodes in gastric lesions.

AUTHOR'S OBSERVATIONS

In the general study of postmortem lymphatic tissue, the outstanding feature was the extreme variability of the structures that might be considered "normal." The variation in lymph nodules was most striking. Secondary nodules of the active type, that is, the type described by Fleming, were exceedingly rare in persons over 30 years of age in the absence of long-standing, mild, chronic inflammatory processes.

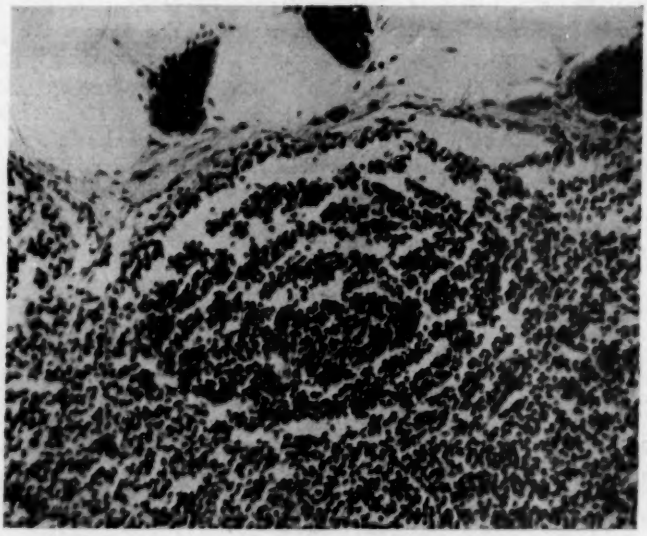

Fig. 1.—A primary lymphatic nodule without a secondary nodule; × 225.

Lymph nodules without secondary nodules were frequently seen in lymph nodes. The necrotic type of secondary nodule was occasionally found, but by far the greater number of secondary nodules were of the hyaline, reticular and epithelioid types. All of the secondary nodules seen could be designated according to the classification for those of the spleen by Groll and Krampf [41] and for those of lymph nodes by Rotter.[52] The various types of secondary nodules are pictured in figures 1, 2, 3, 4, 5, 6 and 7. The rarity of the active type and the abundance of the inactive type of secondary nodule in the age group beyond 30 years of age agree with the findings of Groll and Krampf [41] and Parodi [28] for the spleen, and with those of Meek [74] for lymph nodes.

74. Meek, W. O.: Quart. J. Med. **3**:395, 1910.

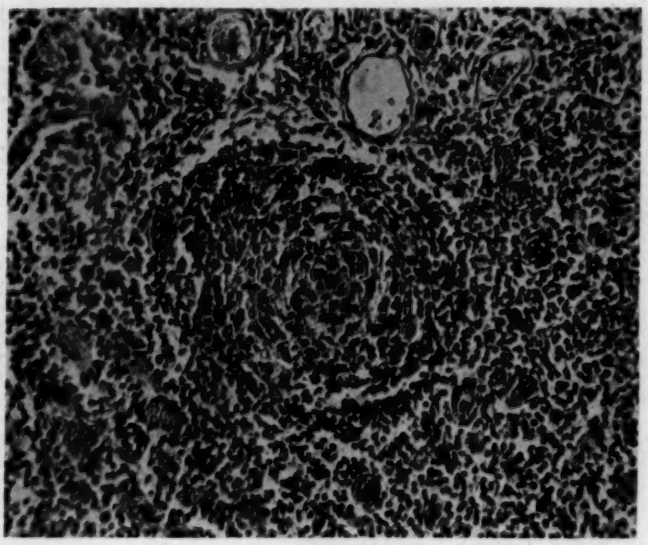

Fig. 2.—A lymphatic nodule with a secondary nodule of the active type; × 200.

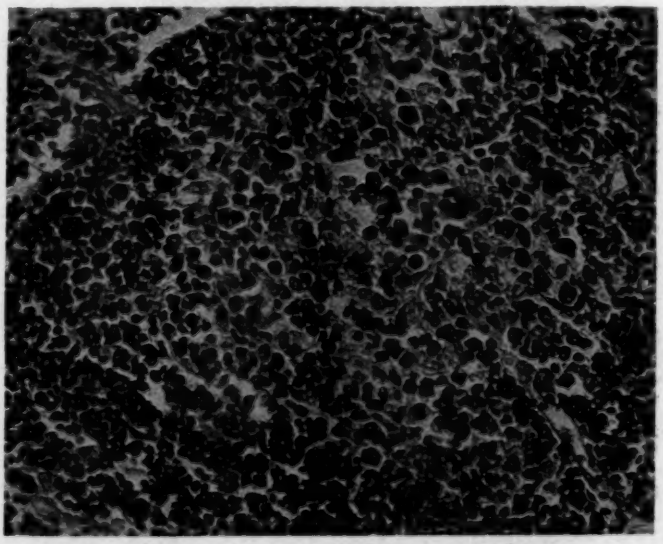

Fig. 3.—The active type of secondary nodule; × 300.

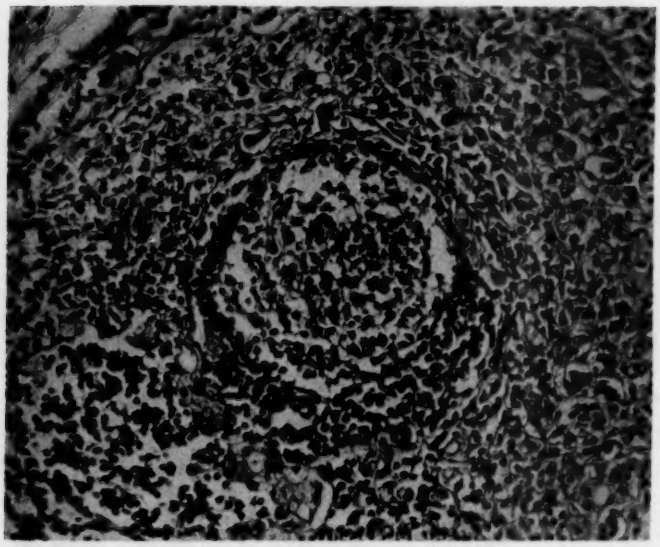

Fig. 4.—A lymphatic nodule with a secondary nodule of the reticular type. The tissue about the nodule is carcinoma; × 250.

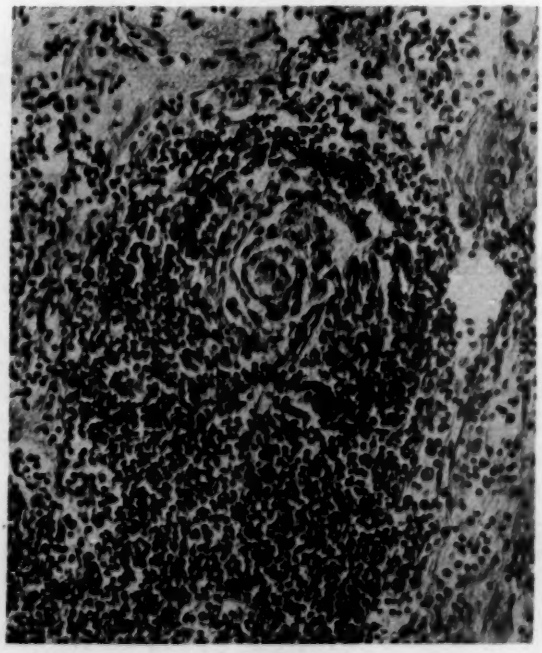

Fig. 5.—A lymphatic nodule with a secondary nodule of the epithelioid type; × 215.

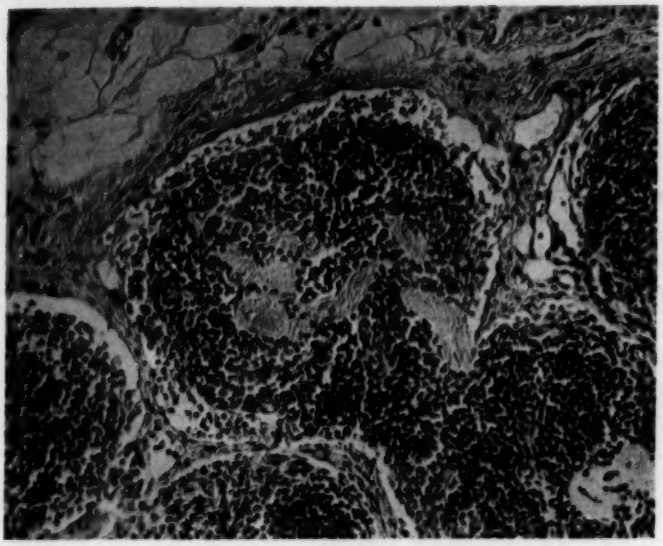

Fig. 6.—A lymphatic nodule with a secondary nodule of the hyaline type; × 215.

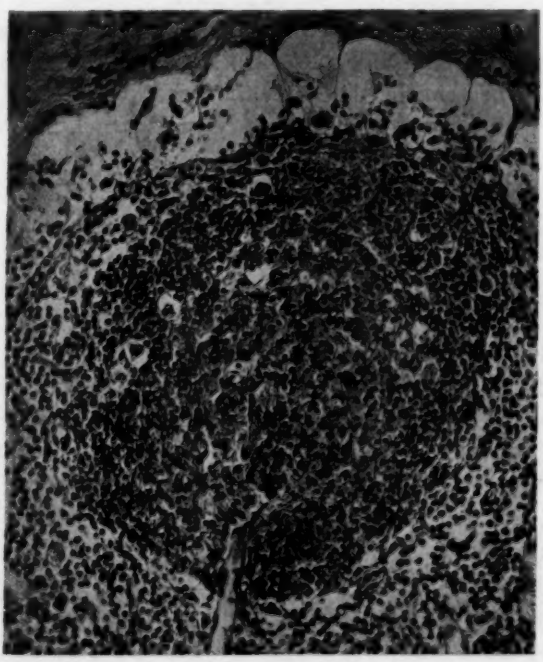

Fig. 7.—A lymphatic nodule with a secondary nodule of the necrotic type; × 275.

The cases for special study were divided into 3 groups as indicated in table 1. Group 1 consisted of 55 cases of carcinoma of the stomach in which 269 lymph nodes were obtained. This group was studied particularly for the case incidence of the active type of secondary nodule. The number of nodes in each case varied from 1 to 12; the average number was 5. In all but one case of this group there were found well formed secondary nodules of the active type. In many of the cases, all the nodes secured contained these secondary nodules. In the cases from which many nodes were secured, there was usually one or more nodes that did not contain the active type of secondary nodule, but did contain primary nodules without secondary nodules, or secondary nodules of the reticular, hyaline or epithelioid type. The case in which no secondary nodules of the active type were found yielded 6 nodes containing the reticular type of secondary nodule. No explanation for this peculiarity could be found.

Group 2 consisted of 43 cases of gastric carcinoma and was selected for an analysis of the incidence of secondary nodules of the active type in each case. This group was suitable for such an analysis because all the lymph nodes resected were available for study. The number of nodes not obliterated by carcinoma in each case varied from 1 to 17; the average number was 6.3. All but 1 of the 43 cases yielded nodes containing secondary nodules of the active type. This case yielded but a single node for study. Of the total of 270 nodes obtained, 138 contained large secondary nodules of the active type. Eighty-two of the nodes contained smaller, but none the less typical, secondary nodules of this type. In 50 of the nodes no secondary nodules of the active type were found.

Group 3 consisted of 18 cases of gastric ulcer, in which 54 nodes were obtained from the resected portions of the stomachs. This group was studied as a control on the cases of carcinoma. All of the cases in this group yielded nodes containing large, well formed secondary nodules of the active type. In most of the cases, all the nodes examined contained this type of secondary nodule. The only difference between this group and that of cases of carcinoma was the smaller number of nodes obtained. This might be expected because of the smaller lesions in ulcer. The number of nodes in each case varied from 1 to 8; the average number was 3.

The histologic and cytologic changes were essentially the same in the three groups, and they will be described together. Changes in the gastric wall were strikingly uniform qualitatively. Sections from the border and the base of all the carcinomas and ulcers showed varying degrees of infiltration with plasma cells and eosinophils. The plasma cell infiltration was most marked in the mucosa and submucosa about

TABLE 1.—*Relationship of Secondary Nodules of Active Type in Regional Lymph Nodes to Gastric Lesions*

Group	Number of Cases	Average Age of Patients, Years	Number of Nodes	Number of Cases with Secondary Nodules of Active Type
1. Carcinoma	55	57.6	269	54
2. Carcinoma	43	54	270	42
3. Ulcer	18	57	54	18
Totals	116		593	114
Average age		56		

TABLE 2.—*Incidence of Nodes Containing Active Secondary Nodules in Group 1, Cases of Gastric Carcinoma in Which Stomachs Were Resected*

Case	Age of Patient, Years	Sex	Number of Nodes	Number of Nodes Containing Secondary Nodules of Active Type	Number of Nodes Without Secondary Nodules of Active Type
1	52	F	5	5	0
2	54	F	6	5	1
3	54	M	7	5	2
4	72	M	3	3	0
5	55	M	12	8	4
6	75	M	2	2	0
8	65	M	6	5	1
9	64	M	3	1	2
10	76	M	1	1	0
11	50	M	7	5	2
12	58	M	10	7	3
13	65	M	4	4	0
14	58	M	1	1	0
15	54	M	1	1	0
16	66	M	2	2	0
17	69	M	1	1	0
18	60	M	11	10	1
19	54	M	4	4	0
20	52	M	6	5	1
21	55	M	3	3	0
22	57	F	4	4	0
23	55	M	4	4	0
24	40	M	9	7	2
25	71	M	2	2	0
26	44	M	2	2	0
27	56	M	2	2	0
28	36	M	7	6	1
29	64	M	9	6	3
30	57	M	2	2	0
31	57	M	1	1	0
33	54	M	10	6	4
34	56	F	3	3	0
35	55	M	8	4	4
36	57	M	1	1	0
38	62	M	1	1	0
39	63	M	1	1	0
40	53	M	1	1	0
41	60	M	10	5	5
42	49	M	6	6	0
43	63	M	6	5	1
44	50	M	10	8	2
45	43	M	4	4	0
46	57	M	2	2	0
47	59	M	3	3	0
48	63	M	9	8	1
49	62	M	1	1	0
50	57	M	12	10	2
51	63	M	10	2	8
52	64	F	4	4	0
53	62	M	9	9	0
55	42	F	8	5	3
56	64	M	5	5	0
57	53	M	6	0	6
58	61	M	1	1	0
59	67	F	1	1	0

TABLE 3.—*Incidence of Nodes Containing Active Secondary Nodules in Group 2, Cases of Gastric Carcinoma in Which Stomachs Were Resected*

Case*	Number of Nodes Containing Lymphoid Tissue	Number of Nodes Containing Secondary Nodules		Number of Nodes Containing No Secondary Nodules
		Large	Small	
1	17	5	6	6
2	9	3	4	2
3	9	8	0	1
4	4	3	1	0
5	3	2	1	0
6	7	0	2	5
7	1	1	0	0
8	4	4	0	0
9	6	5	0	1
10	2	2	0	0
11	10	6	4	0
12	1	1	0	0
13	1	0	0	1
14	4	1	0	3
15	10	3	5	2
16	5	4	1	0
17	6	1	3	2
18	7	7	0	0
19	10	9	1	0
20	6	4	2	0
21	4	2	0	2
22	9	7	2	0
23	7	2	2	3
24	6	4	2	0
25	3	2	0	1
26	6	2	3	1
27	8	2	3	3
28	3	1	1	1
29	8	2	4	2
30	1	1	0	0
31	6	0	5	1
32	4	3	1	0
33	4	3	1	0
34	11	5	3	3
35	8	3	4	1
36	8	2	4	2
37	11	1	9	1
38	10	7	1	2
39	7	4	1	2
40	7	5	1	1
41	3	2	1	0
42	4	0	2	2
43	10	7	3	0
	270	136	83	51

* All 43 cases had nodes containing secondary nodules of the active type.

the borders of the ulcer, whether it was benign or malignant. In many of the cases there were accumulations of plasma cells throughout the muscularis. The eosinophils were present in large numbers throughout the immediate region of the ulcerated portion of the lesion. There were few polymorphonuclear neutrophilic granulocytes in most of the lesions. Three of the carcinomas were of the nonulcerated polypoid type; sections of two of these were marked by a greater number of polymorphonuclear neutrophils than the ulcerating lesions. Plasma cells and eosinophilic granulocytes were also present in these sections.

TABLE 4.—*Incidence of Nodes Containing Active Secondary Nodules in Group 3, Cases of Gastric Ulcer in Which Stomachs Were Resected*

Case	Age of Patient, Years	Sex	Number of Nodes	Number of Nodes Containing Secondary Nodules of Active Type	Number of Nodes Without Secondary Nodules of Active Type
1	38	M	2	2	0
3	67	M	6	6	0
4	66	M	4	4	0
5	65	M	1	1	0
6	46	F	8	5	3
8	44	M	2	2	0
9	56	M	1	1	0
10	64	M	3	3	0
11	61	M	2	2	0
12	53	M	6	6	0
13	66	M	6	3	3
14	65	M	1	1	0
16	57	M	1	1	0
18	67	M	2	2	0
19	61	M	3	3	0
20	26	F	1	1	0
22	58	M	4	4	0
23	65	M	1	0	1
18 cases.....			54	47	7

No definite quantitative correlation between the changes in the gastric wall and the changes in the nodes could be established. But such a relationship was suggested by case 9 in group 1, which was that of a polypoid carcinoma with little chronic inflammatory reaction, case 6 in group 2 and case 18 in group 1, which showed little chronic inflammatory reaction. The nodes from these cases contained few small secondary nodules of the active type; a larger proportion of the nodes than in other cases contained no secondary nodules. Case 58 in group 1 similarly suggests a quantitative relationship. The nodes in this case were numerous and contained unusually large and numerous secondary nodules of the active type (fig. 9). The gastric mucosa and submucosa in this case contained unusually large numbers of plasma cells (fig. 10).

The changes in the lymph nodes containing secondary nodules of the active type were also qualitatively uniform. In group 2, 81.5 per cent of the nodes not completely obliterated by carcinoma contained secondary nodules of the active type. These nodes were characterized also by the presence of many plasma cells in the capsules, sinuses and lymphatic cords. All of the secondary nodules of the active type in all cases were marked by the presence of mitotic figures and active phagocytes. The size of the secondary nodules was directly proportional to the number of mitotic figures and active phagocytes within the secondary nodules. Acute inflammatory reaction, as evidenced by the presence of neutrophilic polymorphonuclear granulocytes, was rarely found. In the presence of more than a few polymorphonuclear leukocytes in the node, secondary nodules of the active type were not found. A post-

Fig. 8.—A lymph node from a stomach resected for gastric carcinoma in a man, aged 58. This is the typical node found in both carcinoma and ulcer; × 11.5.

mortem study of the nodes remaining in the region of the stomach after partial gastrectomy indicated that acute inflammatory processes cause a regression of secondary nodules of the active type. Such studies were made in 14 cases (nos. 5, 9, 10, 11, 13, 20, 29 and 31 in group 1 and nos. 1, 3, 4, 5, 9 and 10 in group 3). In but 2 of these cases (nos. 1 and 9, group 3) were gastric nodes secured post mortem that contained well preserved secondary nodules of the active type. Sections of the stomach from case 9, group 3, contained no evidence of acute inflammation. The patient in case 1, group 3, died of hemorrhage shortly after operation. Nodes in all the other cases of this group contained secondary nodules of various types other than the active. The most common type was the solid nodule. The next most frequent type was the epithelioid, then the hyaline. No necrotic types were found. The interpretation of these findings might be either that

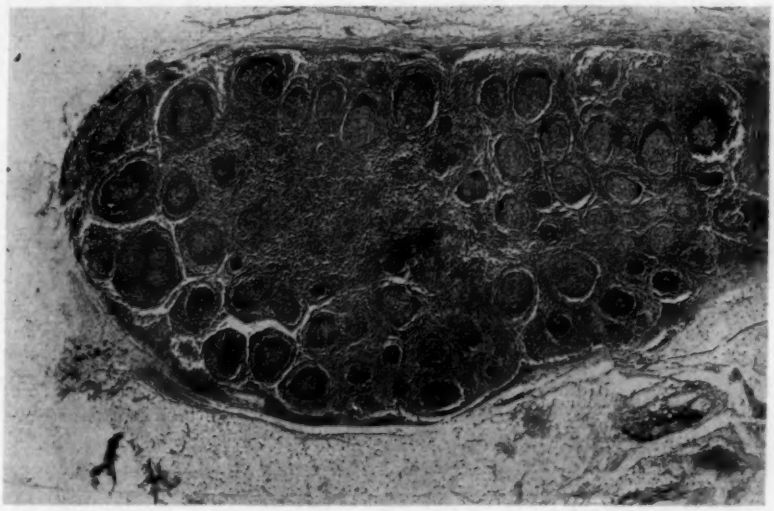

Fig. 9.—A lymph node from a stomach resected for gastric carcinoma in a man, aged 61. The secondary nodules of the active type are extremely large and numerous; × 11.5.

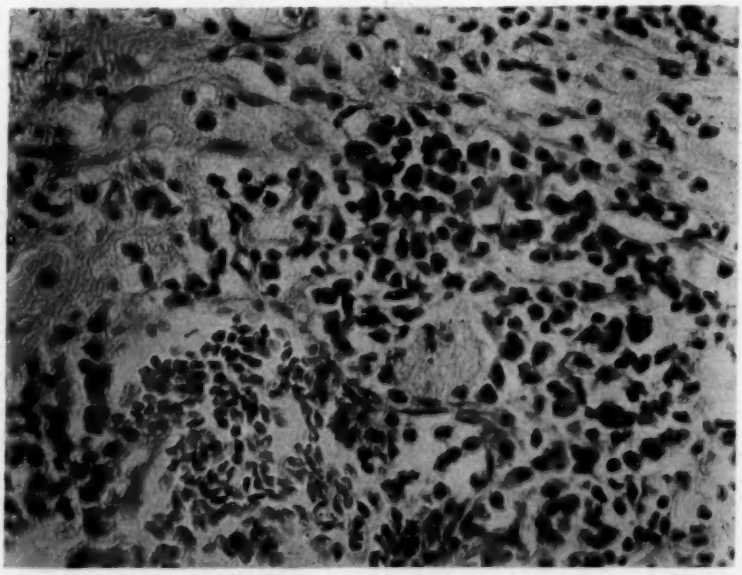

Fig. 10.—A section from the gastric wall in the same case as the lymph node shown in figure 9. Note the plasma cell infiltration; × 400.

the nodes secured post mortem did not drain the original lesion, or that the acute inflammatory reaction resulting from the operation caused a regression of the secondary nodules. No correlation could be established between the degree of fibrosis or of lymphocytic infiltration of the lesion and the reaction in the lymph nodes.

SUMMARY

An examination of 1,500 lymph nodes from persons dying of various causes revealed few secondary nodules of the active type in the age group beyond 30 years. Examination of 592 lymph nodes from the portions of 116 stomachs resected from carcinoma and benign ulcer revealed that 114 (98.2 per cent) of the specimens contained lymph nodes with secondary nodules of the active type. The average age of the persons in this group was 56 years. In a group of 43 cases, 81.5 per cent of the nodes not completely obliterated by carcinoma contained active secondary nodules. Microscopically, the gastric lesions were characterized by an infiltration with plasma cells and eosinophilic leukocytes. The lymph nodes were characterized by the presence of plasma cells in increased numbers and by the presence of well formed secondary nodules containing many mitotic figures and active phagocytes.

COMMENT

Flemming's observations were made on a small amount of material that permitted him to draw but one conclusion. He observed apparently but one type of secondary nodule, that is, the type that he called physiologically a "germinal center." Some subsequent observers failed to substantiate Flemming's findings because they used limited material of a different nature. Since there has been abundant demonstration that there exist several types of secondary nodule, different in structure and different in significance, the designation of all these types of secondary nodule by a single term implying a single significance is no longer justified. If the material used by various workers is considered, many of the apparent conflicts in the literature disappear. There remain, however, several objections to the acceptance of Flemming's theory.

The contention that the secondary nodules of lymphatic tissue are the chief places for the formation of lymphocytes must be denied on the basis of the following facts: 1. Lymphocytes are formed abundantly in the absence of secondary nodules. 2. Lymphocytes are invariably present before secondary nodules are formed. 3. Lymphocytes are formed elsewhere than in secondary nodules at times of life when secondary nodules may be present.

The contention that the secondary nodules of lymphatic tissue are physiologically places for the formation of lymphocytes must be questioned on the basis of the strong presumptive evidence in the literature that the secondary nodules of the active type arise at a time when, and in locations where, pathologic stimuli cannot be eliminated as a factor. Furthermore, there is evidence that the secondary nodules of the active type can be increased in number and size by pathologic stimuli.

The contention that secondary nodules of the active type give rise to lymphocytes must be admitted on the basis of the careful cytologic studies of the hematologists.

CONCLUSIONS

Secondary nodules of the active type ("germ centers," the Flemming type of secondary nodule) are rarely found in lymph nodes of man beyond 30 years of age in the absence of long-standing, mild, chronic inflammatory processes.

Secondary nodules of the active type are common (98.2 per cent of cases) in lymph nodes associated with chronic ulcerating lesions of the stomach in man beyond 30 years of age.

This circumstantial evidence and the histologic picture indicate an etiologic relationship between the pathologic process in the gastric wall and the production of secondary nodules of the active type in the near-by lymph nodes.

This evidence is offered in support of the contention that the secondary nodules of lymph nodes arise as the result of pathologic processes.

These observations on human material support the conclusion drawn from experimentation on animals, that the secondary nodules of lymphatic tissue are reaction centers, and should increase the skepticism with which Flemming's hypothesis is viewed.

EXPERIMENTALLY PRODUCED FOCAL (DENTAL) INFECTION IN RELATION TO CARDIAC STRUCTURE *

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AND
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To our knowledge there has been no report in the literature of an experimentally controlled attempt to correlate the effect of focal infection with cardiac hypertrophy or to determine myocardial response to toxic injury and infection under stress and strain. Rosenow and Meisser [1] reported the experimental production of renal stones in dogs following the establishment of a permanent focal infection in the canine teeth of the animals with a micro-organism which they believed to possess a selective affinity for renal tissue. The method appealed to us as a possible means of studying experimentally the relation, if any, that may exist between chronic focal infection and disease of the heart.

In this paper, we discuss the following problems: (1) the relationship, if any, between experimentally produced focal (dental) infection and cardiac hypertrophy in dogs; (2) the relative effects of exercise on the hearts of such infected and noninfected dogs; (3) the reliability of the various means of expressing cardiac hypertrophy, and (4) other pathologic changes, cardiac and extracardiac, found in the experimental animals.

The literature on the cause of cardiac hypertrophy is voluminous, and no attempt to review it is made here. A few of the principles and opinions concerned are alone noted. Külbs,[2] in reviewing the literature, found a general acceptance of the statement that a work hypertrophy exists, and concluded that an increase in the relative and in the absolute weight of the heart occurs with exercise, without a corresponding increase in the weight of the body muscle. Horvath [3] denied that hypertrophy is due to increased work. Albrecht [4] found no constant

* Submitted for publication, June 24, 1931.

* From the Department of Medicine, University of Oregon Medical School.

1. Rosenow, E. C., and Meisser, J. G.: Arch. Int. Med. **31**:807, 1923.

2. Külbs: Arch. f. exper. Path. u. Pharmakol. **55**:288, 1906.

3. Horvath, A.: Ueber die Hypertrophie des Herzens, Leipzig, W. Braunmüller, 1897; quoted by Stewart (footnote 5).

4. Albrecht, E.: Der Herzmuskel und seine Bedeutung für Physiologie, Pathologie und Klinik des Herzens, Berlin, Julius Springer, 1903; quoted by Stewart (footnote 5).

relation between function and hypertrophy. There is, however, he said, an increase in the connective tissue stroma, and the vacuolated muscle cells show degeneration of the anisotropic portions and increase in the isotropic portions of the muscle elements, with, often, a doubling of the nuclei. Albrecht considered such changes a response to infection. Stewart [5] stated that hypertrophy induced by production of aortic insufficiency involves all chambers of the heart. The hypertrophy is greatest absolutely in the left ventricle, and is next greatest, in order, in the septum, right ventricle and auricles. The greatest relative increase is also seen in the left ventricle, but the auricle shows a greater relative increase than either the septum or the right ventricle. Herrmann [6] concluded that the left ventricle has the greatest relative and the greatest absolute hypertrophy in experimental aortic insufficiency. He stated, further, that older dogs are often refractory to hypertrophy, and that young dogs show more uniformity of response; that the presence or the absence of intracardial infection is an important factor, and that the solution of the problem of cardiac hypertrophy in general is still out of reach because, presumably, a number of factors are active in the process. Goldberger [7] found that the muscle cell of the hypertrophied heart is larger than normal, and stated that the increase is a volume increase. Tangl,[8] after using a maceration process on specimens from the left ventricular wall, concluded that in physiologic growth as well as in pathologic hypertrophy the greater the absolute weight of the heart the greater is the cross diameter of the cells.

EXPERIMENTAL TECHNIC

The dogs used in these experiments constituted an unselected group, except that the age of each approached 9 months, in order that the canine teeth might be sufficiently developed to permit of carrying on this work and the hearts not refractory to infection, and also so that they, in general, might represent an average group comparable to the normal canine standards determined by Herrmann.[9] Thirty-one dogs, divided into three groups, were used.

The twelve dogs of the inoculated group were subjected to a rigidly followed operative technic by Dr. Frank Mihnos, the attending oral surgeon of the Multnomah County Hospital, which may be summarized as follows:

5. Stewart, H. A.: J. Exper. Med. **13**:187, 1911.
6. Herrmann, G. R.: Am. Heart J. **1**:485, 1926.
7. Goldberger, B.: Virchows Arch. f. path. Anat. **103**:88, 1886; quoted by Tangl (footnote 8).
8. Tangl, Franz: Virchows Arch. f. path. Anat. **116**:432, 1889.
9. Herrmann (footnote 6, p. 213).

The dog was anesthetized with ether; the lower canine tooth, or teeth, was isolated with rubber dam and the parts swabbed with iodine. The distal half of the tooth was removed with dental saw and rongeur forceps. The pulp was then removed, the bleeding stopped, and the cavity dried. The cavity was then filled with a streptococcus culture and a pulp point put in place and covered with a small pledget of sterile cotton; a dental cement covering was then placed over the cotton and the tooth permanently filled with silver amalgam.

The streptococcus culture used was isolated by Dr. Benson, of the department of pathology, from antral tissues removed from a patient suffering with chronic hyperplastic sinusitis. It had been previously used by him in an experimental attempt to produce arteriosclerosis in monkeys. The organism was identified by cultural and microscopic characteristics as a nonhemolytic, green-producing, gram-positive type of streptococcus, corresponding in its sugar fermentations to Streptococcus mitis. We chose it because, in Dr. Benson's experiments, the hearts of his inoculated animals showed patchy degenerative changes of the myocardium, and we hoped that it might have a selective action on the heart muscle of our dogs. The injected material was a twenty-four hour culture of this organism, washed in physiologic solution of sodium chloride.

The twelve dogs of the control group were selected as the others had been and were subjected to the same care and treatment with the exception of not being inoculated.

The normal dogs, seven in number, were likewise of the same age. They were secured after death and were weighed, and the fresh hearts, weighed, were kept for further study. This series was started with the idea of continuing the inclusions to an indefinite number. When it was found, however, that the series corresponded, practically without exception, to the large group of two hundred normal dogs reported on by Herrmann,[9] it was discontinued.

The inoculated and the control dogs were subjected to stress and strain by daily exercise to exhaustion. To provide such exercise, an electric treadmill, having an inclined canvas belt and two screened cages, was constructed. Each dog was exercised for a period of fifteen minutes daily, six days per week. The tread inclined at an angle of 20 degrees to the horizontal and moved at a rate of 5.4 miles per hour. The average energy thus expended by each dog in his daily run was 22,800 foot-pounds, and reached the sum total of approximately 3,000,000 foot-pounds during the entire experimental period, which averaged 1,550 minutes of exercise per dog. The daily exercise was sufficient to fatigue each dog markedly. The amount of exercise needed to bring about fatigue varied, for especially did the inoculated dogs show exhaustion more quickly than the control dogs.

The dogs were divided into five groups and placed in pens, each provided with an ample outdoor runway and a steam-heated room, maintained at approximately 65 F. The ration provided was a balanced commercial food product (Kibbled Cakes). Hamburger steak was fed at frequent intervals. Water was constantly provided in the pens. Carbon tetrachloride was used as indicated for tapeworm infestation on diagnosis. The dogs were washed at intervals of a fortnight. Every effort was put forth to keep the dogs in the best possible physical condition during the entire course of the experiment. The animals were weighed before running, at intervals during the course of the experiment, and at death.

METHODS OF EXAMINATION

Roentgenograms of the teeth of the inoculated and of the control series of dogs, made at intervals, informed us of the presence and progress of the dental infections. Postmortem examinations of the jaws for infected areas were made.

Animals dying during the course of the experimentation were subjected to the same postmortem treatment as animals killed by asphyxia during deep ether anesthesia at the conclusion of the experimental period. An immediate necropsy of the thoracic and abdominal viscera was made, and the presence or the absence of gross pathologic changes noted. The upper and lower jaws were saved for dissection and further investigation. The heart was removed from the pericardial sac and prepared according to the method of Lewis.[10] This method may be summarized as follows:

1. The parietal pericardium was removed. 2. The vessels were cut short. 3. The cavities were washed free of clots and drained. 4. The heart was weighed. 5. The vessels were ligated. 6. The cavities were distended with dilute solution of formaldehyde U. S. P. (1:10) injected through the cardiac wall with a hypodermic needle. 7. The heart was immersed in dilute solution of formaldehyde (1:10) for from five to seven days, depending on the size. 8. The orifices were opened, and the heart was drained and washed in running water for from one to two days. 9. The heart was placed in 70 per cent alcohol until its weight reached normal or until a maximum weight that was still less than normal was reached. 10. The epicardium, subepicardial fat, coronary vessels, valves and chordae tendineae were removed. 11. The auricles and ventricles were separated. 12. The cleaned ventricles were separated into left ventricular, right ventricular and septal portions by a series of cuts parallel to and tangential to the septum. 13. The auricles were cleaned. 14. The heart portions were placed in water and drained. 15. The heart portions were weighed individually.

Estimation of Cardiac Hypertrophy.—We investigated four commonly used methods of estimating cardiac hypertrophy in order to adjudge the relative cardiac effects of our experiments on inoculated

10. Lewis, Thomas: Heart **5**:367, 1913-1914.

and noninoculated dogs. These methods of expressing cardiac hypertrophy were:

1. Ratios as follows:
   A. Fresh heart weight to body weight at death
   B. Fixed and prepared total ventricular weight to body weight at death
   C. Fixed and prepared left ventricular weight to body weight at death
   D. Fixed and prepared right ventricular weight to body weight at death
   E. Fixed and prepared septal weight to body weight at death
   F. Fresh auricular weight to body weight at death
2. Ratio of fresh heart weight to body area at death
3. Ratio of fixed and prepared left ventricular weight to right ventricular weight
4. Direct measurement of the diameters of cardiac muscle fibers, with the use of a filar micrometer, from
   A. The apex of the left ventricle (paraffin sections)
   B. The lower one fourth of the interventricular septum (paraffin sections)
   C. Macerated, teased and stained specimens of the apex of the left ventricle

Statistical Analysis.—We made the following statistical computations:

1. Frequency polygons, showing for both inoculated and control series:
   A. Ratio of heart weight to body weight at death
   B. Ratio of fixed and prepared total ventricular weight to body weight at death
   C. Ratio of fixed and prepared left ventricular weight to body weight at death
   D. Ratio of fixed and prepared right ventricular weight to body weight at death
   E. Ratio of fixed and prepared septal weight to body weight at death
   F. Ratio of estimated fresh auricular weight to body weight at death
   G. Average diameter of cardiac muscle fibers from paraffin sections of apex of left ventricle
   H. Average diameter of cardiac muscle fibers from paraffin sections of lower one fourth of interventricular septum
   I. Average diameter of cardiac muscle fibers from macerated specimens of apex of left ventricle
   J. Average diameter of cardiac muscle fibers based on the sum of all microscopic muscle fiber diameter measurements
2. Scatter diagrams for both inoculated and control series between the following variables:
   A. Ratio of fresh heart weight to body weight at death in relation to
      a. Total exercise time in minutes
      b. Ratio of fresh heart weight to body surface area

c. Body weight at death
d. Fresh heart weight
e. Average muscle fiber diameter

B. Total exercise time in relation to
a. Average muscle fiber diameter
b. Ratio of fresh heart weight to body surface area

C. Ratio of fresh heart weight to body surface area in relation to
a. Fresh heart weight
b. Body surface area
c. Average muscle fiber diameter

3. Linear step-interval distribution between inoculated and control groups showing percentage deviation from average values of the same units in the control series:

A. Ratio of fresh heart weight to body area at death
B. Ratio of fresh heart weight to body weight at death

4. Pearson product-moment coefficient of correlation between:

A. Fixed and prepared total ventricular weight and body weight at death
B. Fixed and prepared left ventricular weight and body weight at death
C. Fixed and prepared left ventricular weight to right ventricular weight
D. Percentage of body weight lost during experimental period and ratio of fresh heart weight to body weight at death

5. The reliability coefficients of our data were computed by the method of

$$\frac{M_1 - M_2}{\sqrt{(\sigma_{M_1})^2 \ (\sigma)^2_{M_2}}} = \frac{D}{\sigma D}$$

and the obtained results interpreted on a table given by Garrett [11] for the following ratios:

A. Fresh heart weight to body weight at death
B. Fixed and prepared total ventricular weight to body weight at death
C. Fixed and prepared left ventricular weight to body weight at death
D. Fixed and prepared right ventricular weight to body weight at death
E. Fixed and prepared left ventricular to right ventricular weight

6. The ratios of fresh heart weight to body weight for our seven normal dogs were calculated according to the previously described method

The raw data for inoculated, control and normal animals are presented in tables 1, 2, 3 and 4. The column numbers in table 1 represent the following values:

1. Identification number of dog
2. Sex of dog
3. Date of beginning of experimental period

11. Garrett, H. E.: Statistics in Psychology and Education, New York, Longmans, Green & Co., 1926.

4. Body weight at beginning of experimental period
5. Total exercise time in treadmill in minutes
6. Running ability: 1 (best) to 4 (poorest)
7. Death during course (C) of, or end (E) of, experimental period
8. Date of end of experimental period
9. Length of experimental period in days
10. Body weight at end of experimental period
11. Percentage of original body weight lost during experimental period
12. Cause of death
13. Average diameter of muscle fibers—paraffin sections from apex of left ventricle—in microns
14. Average diameter of muscle fibers—paraffin sections from lower quarter of interventricular septum—in microns
15. Average diameter of muscle fibers—teased, macerated, stained fibers from apex of left ventricle—in microns
16. Estimated surface area of dog in square meters
17. Ratio of fresh heart weight at death to estimated area of body surface at death
18. Percentage deviation of the ratio of heart weight to surface area from the control average
19. Ratio of fresh heart weight at death to body weight at death
20. Percentage deviation of the ratio of heart weight to body weight from the control average
21. Ratio of heart weight (fresh) to body weight at beginning of experimental period, with heart weight at death taken as a standard
22. Ratio of heart weight to body weight at beginning of experiment, with normal (Herrmann[9]) heart weight taken as standard
23. Ratio of fixed and prepared left ventricular weight to right ventricular weight
24. Total weight of fresh heart at death in grams
25. Total weight of fixed and prepared heart in grams
26. Ratio of fresh heart weight to body weight at death
27. Total weight of fixed and prepared ventricles in grams
28. Total weight of fixed and prepared ventricles to body weight at death
29. Weight of fixed and prepared left ventricle in grams
30. Ratio of weight of fixed and prepared left ventricle to body weight at death
31. Weight of fixed and prepared right ventricle in grams
32. Ratio of weight of fixed and prepared right ventricle to body weight at death
33. Weight of fixed and prepared septum in grams
34. Ratio of weight of fixed and prepared septum to body weight at death
35. Estimated weight of fresh auricles in grams
36. Ratio of estimated weight of fresh auricles to body weight at death

ESTIMATIONS OF CARDIAC HYPERTROPHY

Method 1 A.—The ratio of mean fresh heart weight to body weight at death of our inoculated series is 0.00883 with a σ of 0.000360; that of our control series, 0.00792 with a σ of 0.000336, and that of normal dogs, as reported by Herrmann,[9] 0.00798 with a σ of 0.0001036. Since the ratios of the control dogs, which were subjected to the same severe and prolonged exercise as the inoculated group, remained an average of but 0.9 per cent below reported normal level values, we are reasonably sure in concluding that the observed difference is due to unavoidable experimental errors, and that severe and prolonged exercise in the absence of toxic injury does not raise the ratio of heart weight to body weight. The inoculated group shows a gain of 10.08 per cent, which indicates a definitely demonstrable gross relative cardiac hypertrophy, according to this method.

Method 1 B, C, D, E, F.—The increased ratio of heart weight to body weight is chiefly due to the increased size of the left ventricle, with the septum, right ventricle and right auricle hypertrophied, as shown in table 5.

The figures for fixed normal auricles are not given by Herrmann,[9] but a normal average is estimated from reported figures for the purpose of this paper. From table 5 it may be seen that the septum and the left ventricle are increased the most, with an apparently smaller involvement of the right chambers of the heart. It is possible that our particular dissection of the right ventricle and septal portions might have included more heart muscle in the septal portion at the expense of the right ventricular weight, a condition that would explain the apparent disproportion between these figures. The ratios quoted, based on the weights of the fresh heart, fixed and prepared total ventricles and left ventricle, are unaffected by this factor and suggest a moderate gross relative cardiac hypertrophy according to this method of expression. These figures, in the main, corroborate the work of Stewart.[5]

Method 2.—On account of the fact that absolute weights of hearts are useless for comparison because of variations in body size, we are forced to relate the heart weights to some measure of body size. There is evidence [12] that, in general, the heart weight, as well as other organ weights, of a group of animals in a similar state of nutrition, but of varying size, shows less variability when expressed as the ratio of heart weight to body surface area than when expressed as the ratio of heart weight to body weight. To investigate this matter, we have calculated

12. MacKay, L. L., and MacKay, E. M.: Am. J. Physiol. **83**:179, 1927.

TABLE 1.—

Group	Dog	Sex	Date Experiment Began	Body Weight When Experiment Began, Kg.	Total Exercise Time, Min.	Running Ability*	Death in Course of or at End of Experiment	Date Experiment Ended	Days of Experiment or of Life of Dog	Body Weight When Experiment Ended, Kg.	Weight Lost, per Cent	Cause of Death
	1	2	3	4	5	6	7	8	9	10	11	12
Inoculated	1	M	12/ 3/27	8.2	2,145	2	C	6/30/28	209	6.8	16.2	Fighting
	2	M	12/ 4/27	10.9	1,539	3	C	8/18/28	256	10.3	5.1	Fighting
	3	M	12/ 4/27	14.3	1,035	2	C	2/12/28	70	11.1	22.3	Acute cardiac failure
	5	M	12/ 4/27	11.8	912	2	C	2/24/28	82	7.9	32.7	Acute cardiac failure
	7	M	12/ 8/27	11.3	2,911	2	C	8/12/28	247	9.3	18.0	Fighting
	10	M	12/ 8/27	9.1	2,422	2	C	7/ 4/28	209	8.4	7.5	Fighting
	16	M	1/10/28	12.4	392	4	C	2/24/28	45	7.7	38.2	Acute cardiac failure
	22	M	7/27/28	9.3	16	3	E	2/23/29	154	9.1	2.5	Experimental
	24	M	7/27/28	4.7	113	3	C	12/ 8/28	134	3.9	17.0	Fighting
	8	F	12/ 8/27	8.1	3,462	3	E	11/ 1/28	329	6.6	19.5	Experimental
	9	F	12/ 8/27	5.6	3,778	1	E	11/ 1/28	329	4.2	28.8	Experimental
	17	F	1/14/28	5.0	835	2	C	4/ 3/28	82	3.4	31.7	Acute cardiac failure
Average				9.28	1,547	2.54			179	7.41	19.9	
Control	6	M	12/ 4/27	15.2	3,861	1	E	11/ 1/28	333	13.2	13.5	Experimental
	13	M	12/ 8/27	11.1	3,575	2	E	11/ 1/28	329	10.9	2.0	Experimental
	20	M	7/27/28	7.0	2,200	2	E	2/23/29	210	8.2	+11.6	Experimental
	25	M	7/27/28	7.7	1,271	2	C	1/11/29	167	7.5	3.0	Fighting
	26	M	7/27/28	6.8	440	4	E	2/23/29	209	7.3	+5.0	Experimental
	27	M	7/27/28	4.7	465	4	C	10/23/28	88	5.6	+12.2	Fighting
	11	F	12/ 8/27	6.6	3,610	3	E	11/ 1/28	330	5.2	20.5	Experimental
	14	F	1/ 9/28	5.9	603	4	E	11/ 1/28	293	4.8	19.1	Experimental
	28	F	11/ 3/28	9.4	830	1	E	2/23/29	111	7.5	20.5	Experimental
	29	F	11/ 3/28	13.0	837	1	E	2/23/29	111	12.5	4.0	Experimental
	30	F	11/ 3/28	11.0	851	2	E	2/23/29	111	8.5	14.8	Experimental
	31	F	11/ 3/28	14.2	829	1	E	2/23/29	111	14.1	1.0	Experimental
Average				8.91	1,612	2.25			202	8.76	4.97	
Endocarditis control	12	M	12/ 8/27	10.0	792	4	C	3/26/28	169	8.2	18.1	Acute cardiac failure

TABLE 1.—*General Data.—Continued*

Group	Dog	Heart: Total Weight When Fresh (at Death), Gm.	Heart: Total Weight When Fixed and Prepared, Gm.	Heart: Ratio Fresh Heart Weight to Body Weight at Death	Ventricles: Total Weight When Fixed and Prepared, Gm.	Ventricles: Ratio Fixed and Prepared Total Weight to Body Weight at Death	Left Ventricle: Weight When Fixed and Prepared, Gm.	Left Ventricle: Ratio Fixed and Prepared Weight to Body Weight at Death	Right Ventricle: Weight When Fixed and Prepared, Gm.	Right Ventricle: Ratio Fixed and Prepared Weight to Body Weight at Death	Septum: Weight When Fixed and Prepared, Gm.	Septum: Ratio Fixed and Prepared Weight to Body Weight at Death	Auricle: Weight When Fresh, Gm. (Estimated)	Auricle: Ratio Fresh Weight to Body Weight at Death
		24	25	26	27	28	29	30	31	32	33	34	35	36
Inoculated	1	57.1	52.0	0.00835	51.8	0.00757	24.0	0.00361	17.0	0.00248	10.8	0.00158	5.15	0.00075
	2	78.5	75.9	0.00762	70.4	0.00684	34.0	0.00329	22.8	0.00221	13.6	0.00132	8.20	0.00079
	3	77.2	52.1	0.00695	67.0	0.00604	36.7	0.00330	16.1	0.00245	14.2	0.00128	10.10	0.00091
	5	83.6	53.2	0.01052	74.9	0.00945	38.0	0.00521	19.9	0.00274	17.0	0.00234	8.61	0.00118
	7	67.4	64.2	0.00725	60.1	0.00646	28.4	0.00306	21.9	0.00236	9.8	0.00105	7.35	0.00078
	10	69.0	68.4	0.00822	62.6	0.00746	29.9	0.00357	18.9	0.00226	13.8	0.00165	6.00	0.00073
	16	59.2	39.3	0.00770	49.0	0.00636	26.3	0.00337	12.5	0.00160	10.2	0.00131	10.00	0.00128
	22	77.5	82.5	0.00855	67.1	0.00740	36.6	0.00404	16.9	0.00186	13.6	0.00150	10.30	0.00114
	24	49.0	42.0	0.01272	38.1	0.00978	17.4	0.00451	11.1	0.00288	9.6	0.00249	3.90	0.00104
	8	50.0	54.8	0.00760	49.7	0.00756	25.5	0.00388	13.3	0.00202	11.0	0.00167	5.00	0.00076
	9	43.5	46.5	0.01040	40.7	0.00974	19.5	0.00465	12.0	0.00286	9.3	0.00221	5.75	0.00137
	17	34.1	27.0	0.01004	30.2	0.00888	14.8	0.00436	8.2	0.00243	7.1	0.00209	3.84	0.00113
Average		63.9	54.8	0.00883	55.1	0.00778	26.8	0.00391	15.9	0.00234	11.7	0.00171	7.01	0.00089
Control	6	106.0	96.8	0.00805	83.6	0.00635	42.8	0.00325	22.8	0.00173	18.5	0.00141	12.80	0.00097
	13	84.1	76.3	0.00772	69.0	0.00633	34.3	0.00314	15.8	0.00145	19.0	0.00174	7.80	0.00071
	20	61.1	67.5	0.00750	53.8	0.00660	26.3	0.00323	14.4	0.00177	13.1	0.00161	7.22	0.00088
	25	66.0	57.8	0.00881	58.0	0.00775	33.7	0.00450	12.9	0.00172	11.4	0.00152	7.98	0.00106
	26	60.5	65.0	0.00835	54.0	0.00744	23.2	0.00320	15.9	0.00219	14.9	0.00206	6.46	0.00089
	27	40.6	38.0	0.00719	33.5	0.00593	18.3	0.00323	7.25	0.00128	8.0	0.00141	4.50	0.00079
	11	35.6	32.4	0.00685	28.4	0.00545	14.6	0.00281	7.75	0.00149	6.0	0.00116	4.00	0.00077
	14	39.0	36.0	0.00818	30.3	0.00635	15.8	0.00320	7.25	0.00152	7.3	0.00152	5.75	0.00120
	28	69.5	77.0	0.00929	62.2	0.00831	28.0	0.00374	18.9	0.00252	15.3	0.00205	7.22	0.00096
	29	106.5	117.0	0.00855	93.9	0.00753	46.4	0.00373	25.5	0.00205	21.9	0.00176	12.70	0.00102
	30	64.5	56.0	0.00759	58.9	0.00693	29.5	0.00458	15.0	0.00232	14.4	0.00224	5.75	0.00089
	31	98.0	91.5	0.00808	83.1	0.00592	40.1	0.00285	22.4	0.00160	20.6	0.00147	8.40	0.00060
Average		69.3	67.6	0.00792	59.0	0.006765	29.4	0.00345	15.1	0.00181	14.2	0.00165	7.53	0.00087
Endocarditis control	12	78.6	75.5	0.00965	71.1	0.00960	35.0	0.00429	19.5	0.00239	16.6	0.00204	7.60	0.00092

* 1 = best; 4 = poorest. † Paraffin sections. ‡ Paraffin sections from lower quarter.

13	14	15	16	17	18	19	20	21	22	23
Average Diameter of Muscle Fiber, Microns										
Apex Left Ventricle†	Interventricular Septum†	Apex Left Ventricle Teased, Macerated, Stained	Surface Area, Sq.M.	Ratio Fresh Heart Weight to Body Area at Death	Percentage Deviation of Ratio 17 from Control Average	Ratio Fresh Heart Weight to Body Weight at Death	Percentage Deviation of Ratio 18 from Control Average	Ratio Fresh Heart Weight to Body Weight at Start, with Heart Weight at Death Taken as Standard	Ratio Heart Weight to Body Weight at Start, with Normal[a] Heart Weight Taken as Standard	Ratio Fixed and Prepared Left Ventricle Weight to Right Ventricle Weight
7.51	7.95	7.99	0.403	141.9	— 1.4	0.00835	5.5	0.00909	0.00951	1.41
7.60	7.49	7.33	0.530	148.0	2.8	0.00762	— 3.8	0.00775	0.00841	1.49
7.45	7.68	7.64	0.556	137.9	— 4.2	0.00695	—12.2	0.00895	0.01028	2.28
7.86	7.31	7.59	0.446	187.3	30.1	0.01052	32.8	0.01360	0.01185	1.91
7.19	7.44	7.44	0.488	138.1	— 4.0	0.00725	— 8.5	0.00885	0.00974	1.30
7.93	8.17	8.06	0.457	151.0	4.9	0.00822	3.8	0.00890	0.00862	1.58
7.56	7.33	7.25	0.435	136.6	— 5.1	0.00770	— 2.8	0.01228	0.01290	2.05
7.01	7.35	6.98	0.478	162.0	12.6	0.00855	8.0	0.00875	0.00820	2.17
7.06	7.59	6.99	0.274	178.9	24.3	0.01272	60.6	0.01310	0.00961	1.57
7.29	7.57	6.83	0.393	127.1	—11.7	0.00760	— 4.1	0.00945	0.00991	1.93
7.32	7.36	7.59	0.291	149.5	3.9	0.01040	31.4	0.01460	0.01120	1.63
7.13	7.05	7.00	0.253	134.9	— 6.3	0.01004	26.7	0.01470	0.01170	1.80
7.41	7.53	7.38	0.417	149.3		0.00883		0.01057	0.01001	1.765
5.48	5.96	5.61	0.623	170.0	18.1	0.00805	1.6	0.00930	0.00923	1.88
6.11	6.63	6.32	0.550	153.0	6.4	0.00772	— 2.5	0.00786	0.00815	2.18
6.78	6.78	6.47	0.453	134.8	— 6.4	0.00750	— 5.3	0.00646	0.00715	1.82
6.58	6.52	6.37	0.427	154.5	7.4	0.00881	11.3	0.00909	0.00823	2.61
6.32	6.60	6.33	0.418	144.7	0.6	0.00835	5.4	0.00795	0.00760	1.46
6.25	6.45	6.00	0.355	114.4	—20.5	0.00719	— 9.2	0.00640	0.00715	2.52
7.36	7.23	7.30	0.336	106.0	—26.4	0.00685	—13.5	0.00861	0.01003	1.88
6.28	6.55	5.82	0.318	122.8	—14.6	0.00818	3.2	0.01021	0.00989	2.18
6.50	6.77	6.51	0.427	162.7	13.0	0.00929	17.3	0.01170	0.01003	1.48
6.18	6.78	5.85	0.601	176.2	22.5	0.00855	8.0	0.00890	0.00832	1.78
6.51	6.40	6.51	0.465	138.8	— 3.5	0.00759	— 4.2	0.00890	0.00938	1.97
6.55	6.78	6.39	0.651	150.3	4.4	0.00698	—11.9	0.00705	0.00806	1.79
6.41	6.62	6.29	0.469	143.9		0.00792		0.00863	0.00860	1.964
7.31	....	....	.....	.....	.....	0.00965	.. ...	0.01180	0.00975	1.80

TABLE 2.—*Diameters of Muscles Fibers from Hearts of Normal Dogs*

	Dog A	Dog B	Dog C	Dog D
Apical[a]	6.85	6.65	6.95	6.00
	6.55	6.80	6.85	6.05
	7.10	6.95	6.85	5.95
	6.80	7.05	6.70	6.50
	6.20	6.45	6.50	5.45
	6.10	6.50	7.10	6.05
	7.05	6.20	7.05	6.20
	6.85	6.50	6.45	6.10
	6.80	6.70	6.65	6.45
	6.60	6.55	7.20	6.05
	6.65	6.75	7.30	6.20
	6.65	6.80	7.10	5.80
	6.35	6.45	7.00	5.90
	6.20	6.55	7.20	6.15
	6.50	6.65	6.60	6.30
	6.10	6.65	6.80	6.35
	6.45	6.75	6.85	5.45
	6.35	7.10	6.65	6.20
	6.70	6.60	6.70	5.85
	6.80	6.50	6.60	6.00
Average	6.58	6.66	6.86	6.05
Teased apical[a]	5.6	5.2	6.9	5.9
	6.6	6.3	6.8	5.8
	6.6	6.6	6.0	6.2
	6.7	6.7	7.1	6.1
	6.3	6.0	5.8	7.1
	6.1	5.6	7.3	6.1
	5.5	7.0	6.6	5.9
	6.2	6.2	5.9	6.0
	6.1	7.5	6.5	6.3
	6.4	6.6	6.3	6.2
Average	6.23	6.37	6.52	6.16

[a] Diameters are expressed in microns.

TABLE 3.—*Diameters of Muscle Fibers from Hearts of Inoculated and Control Dogs*

	Inoculated Group of Dogs												Control Group of Dogs											
	1	2	3	5	7	10	16	22	24	8	9	17	6	13	20	25	26	27	11	14	28	29	30	31
Septal*	9.3	6.3	9.0	8.8	8.8	8.4	8.0	6.3	6.3	7.7	6.5	7.9	5.8	7.0	6.8	6.3	7.4	7.2	6.7	6.4	6.7	7.2	5.7	7.1
	8.1	8.1	7.3	7.3	8.4	8.7	6.9	6.8	7.8	6.8	8.6	7.6	6.4	6.1	7.6	5.4	5.8	7.1	7.2	6.5	7.3	5.4	5.1	6.8
	5.7	8.0	6.6	6.3	7.1	8.9	7.5	7.9	7.7	8.2	8.2	6.4	7.1	6.3	7.1	7.1	6.4	6.4	6.2	6.8	6.5	7.1	6.3	7.3
	8.0	7.3	8.0	7.8	6.9	8.3	7.5	8.4	7.9	7.5	7.2	6.1	6.1	7.2	5.6	6.6	6.5	5.6	7.3	7.2	7.2	6.3	5.6	7.4
	5.8	6.8	8.0	7.2	7.3	7.7	7.9	6.5	7.4	7.6	7.3	7.4	5.4	7.5	6.7	5.3	5.4	7.4	6.0	6.0	8.1	7.6	7.6	5.9
	9.9	6.5	6.2	6.8	7.0	9.3	6.7	7.6	7.0	7.1	7.4	7.2	5.9	5.9	6.8	6.2	7.3	6.1	6.2	6.3	6.5	7.3	7.1	7.6
	7.7	8.3	8.5	7.8	7.5	7.5	7.7	6.9	6.7	6.9	8.0	6.0	5.7	6.4	6.0	7.0	7.3	6.9	7.3	6.9	5.7	7.1	6.8	6.5
	8.3	8.2	8.4	7.0	6.9	7.8	7.0	6.3	8.7	8.9	8.7	6.9	5.6	6.5	6.7	6.5	6.7	6.0	7.6	6.1	6.3	7.5	7.3	7.1
	7.0	7.7	6.8	6.9	7.4	8.1	7.4	8.5	8.3	7.3	5.7	8.0	6.2	6.5	7.7	7.8	6.6	5.6	8.7	7.1	7.3	6.4	6.1	6.1
	9.7	7.7	8.0	7.2	7.1	7.0	6.7	8.3	8.1	7.7	6.0	7.0	5.4	6.9	6.8	7.0	6.6	6.2	8.1	6.2	6.1	5.9	6.4	6.0
Average	7.95	7.49	7.68	7.31	7.44	8.17	7.33	7.35	7.59	7.57	7.36	7.05	5.96	6.63	6.78	6.52	6.60	6.45	7.13	6.55	6.77	6.78	6.40	6.78
Apical*	9.5	5.8	8.4	7.8	7.3	7.1	7.5	7.2	6.6	7.1	6.4	7.0	4.8	5.7	5.1	6.4	6.2	6.2	6.2	6.0	6.0	6.4	5.2	7.2
	7.2	6.7	6.1	7.4	7.7	9.0	7.0	7.4	7.0	7.3	6.5	7.7	5.4	5.4	6.3	6.7	5.6	6.6	7.8	7.6	7.0	5.1	5.4	7.4
	7.5	6.5	6.8	7.9	8.0	8.7	8.1	6.2	6.4	7.3	8.7	6.9	5.9	6.5	6.5	7.4	7.0	6.2	8.7	4.5	5.4	5.8	7.2	6.8
	7.6	8.1	6.4	8.7	8.5	8.0	7.4	7.5	7.1	6.4	6.2	7.1	4.7	6.0	7.2	5.9	6.8	7.0	6.9	6.0	6.6	6.3	7.0	6.2
	6.1	8.0	6.7	7.5	6.9	8.6	7.6	7.3	8.3	6.8	7.5	7.0	5.9	5.0	7.5	7.3	6.6	6.8	6.8	6.5	5.7	6.0	7.6	6.4
	7.4	8.9	8.4	9.1	6.9	7.0	7.8	6.3	7.0	7.0	7.4	6.5	5.9	7.3	6.0	5.8	6.2	5.6	7.0	6.3	6.9	6.8	7.0	5.0
	7.1	9.0	8.7	6.8	7.4	6.1	6.9	7.1	6.2	7.7	6.8	8.2	5.3	7.0	7.2	6.2	6.0	6.8	7.5	5.9	5.9	6.2	6.2	7.5
	8.1	8.0	7.2	8.1	6.2	7.8	8.4	7.2	7.2	8.2	8.6	6.8	5.4	6.0	7.9	7.1	6.6	5.6	8.6	6.5	7.5	7.4	7.2	7.1
	8.2	7.6	7.4	7.7	6.3	9.4	8.2	7.0	6.9	6.9	6.5	7.5	5.4	5.9	6.7	6.3	5.6	5.4	6.3	6.1	6.9	5.6	6.2	5.6
	6.4	7.4	8.4	7.6	6.7	7.6	6.7	6.9	7.9	8.2	8.6	6.6	6.1	6.3	7.4	6.7	6.6	6.3	7.8	7.4	7.1	6.2	6.1	6.3
Average	7.51	7.60	7.45	7.86	7.19	7.93	7.56	7.01	7.06	7.29	7.32	7.13	5.48	6.11	6.78	6.58	6.32	6.25	7.36	6.28	6.50	6.18	6.51	6.55
Teased apical*	7.0	7.2	7.4	8.2	7.3	7.0	6.3	7.6	7.8	8.2	7.2	7.0	5.9	5.9	6.2	6.2	6.8	6.2	6.4	5.3	6.9	4.9	5.3	6.6
	8.4	7.0	8.5	7.8	8.1	8.3	7.0	7.9	6.0	6.8	7.6	6.6	5.4	6.1	7.3	6.2	6.3	7.0	6.7	5.7	5.8	5.7	6.9	7.1
	7.7	6.8	7.2	8.4	8.1	8.6	7.3	7.0	6.6	7.4	6.7	6.3	5.9	5.9	5.9	6.3	6.7	6.6	8.2	6.5	6.3	6.2	6.8	6.7
	7.3	7.0	7.7	8.4	7.8	8.5	6.9	6.9	6.9	5.9	7.3	7.8	5.4	6.6	6.7	5.8	6.1	6.1	7.5	6.1	6.5	6.2	7.1	6.7
	7.8	8.0	6.7	8.5	7.1	7.5	7.6	8.0	7.2	7.0	7.8	6.8	5.3	5.6	6.5	6.4	6.5	6.3	7.2	5.8	6.8	5.7	6.6	6.4
	7.9	7.6	8.2	6.6	7.0	8.2	6.7	6.5	7.5	6.0	8.0	6.6	6.1	6.5	6.7	6.6	6.3	5.1	6.9	5.1	6.1	5.9	7.2	6.1
	8.8	7.8	7.1	7.2	6.5	8.7	7.1	7.3	7.1	6.2	7.9	7.5	4.7	5.9	6.1	5.7	6.8	6.6	7.6	5.5	6.9	5.8	6.8	6.5
	9.4	7.6	7.4	7.0	7.6	7.3	8.0	6.1	6.9	7.1	8.1	7.1	6.4	6.9	6.8	7.6	5.6	5.3	7.4	6.8	6.6	7.1	6.0	5.9
	8.1	7.0	8.8	6.9	7.1	9.0	8.0	6.2	7.6	6.6	8.0	7.3	6.2	6.8	5.8	5.6	6.2	5.3	6.9	6.3	5.9	5.3	6.2	6.0
	7.6	7.3	7.4	6.9	7.8	7.5	7.6	6.3	6.5	7.1	7.2	7.0	4.8	7.0	6.7	7.3	6.0	5.5	8.2	5.1	7.3	5.7	6.2	5.9
Average	7.99	7.33	7.64	7.59	7.44	8.06	7.25	6.98	6.99	6.83	7.58	7.00	5.61	6.32	6.47	6.37	6.33	6.00	7.30	5.82	6.51	5.85	6.51	6.39

* Diameters are expressed in microns.

TABLE 4.—*Data on Heart Weight of Normal Dogs*

Dog	Body Weight, Kg.	Fresh Heart Weight, Gm.	Ratio Fresh Heart Weight to Body Weight at Death
A	11.3	86.1	0.00761
B	6.3	46.2	0.00733
C	9.8	79.5	0.00810
D	7.5	58.5	0.00780
E	10.0	77.6	0.00776
F	9.2	72.0	0.00781
G	5.15	41.4	0.00802
Average	8.47	66.0	0.00776

body surface area for each of the dogs according to the formula [13] and have calculated the corresponding ratios of heart weight to body area:

$$\text{Body Area} = (\text{Body Weight})^{0.667} \text{ times } 0.112$$

Group	Body Area, Sq. M.	Ratio of Heart Weight to Body Area	Percentage Above Control
Inoculated	0.417	149.3	3.9
Control	0.469	143.9	

Percentage deviation of unit figures from control averages, as suggested by Dr. Thomas Addis,[14] are as follows:

Group	Ratio of Heart Weight to Body Area	Ratio of Heart Weight to Body Weight
Inoculated	+ 2.96 per cent	+11.20 per cent
Control	+ 0.38 per cent	+ 0.51 per cent

These results indicate that the heart weight of the inoculated series is less variable when expressed as the ratio of heart weight to body area than when expressed as the ratio of heart weight to body weight, although the number of experimental animals used is too small to permit such a conclusion.

Method 3.—The ratios of the weight of the fixed and prepared left ventricle to that of the right ventricle are found to be:

Group	Ratio of Left Ventricle to Right Ventricle	Percentage Above Normal
Inoculated	1.765 σ 1.79	20.9
Control	1.964 σ 1.99	34.2
Normal	1.461 σ 0.317	

13. Rogers, C. G.: Textbook of Comparative Physiology, ed. 1, New York, McGraw-Hill Book Company, 1927, p. 357.

14. Addis, Thomas: Personal communication.

Since the probable errors of the inoculated and of the control group are so large in comparison to the means of these values, relatively little credence can be placed in the apparent increase over the normal values other than as indicating a general tendency.

Method 4 A, B, C.—Direct measurement of the diameters of cardiac muscle fibers with the use of a filar micrometer and oil immersion lens on stained specimens gives evidence of a microscopic cardiac hypertrophy. The data are shown in tables 2 and 3. The results may be summarized as follows:

Group	Diameter of Muscle Fiber, Microns	Percentage of Normal
Inoculated	7.44	115.0
Control	6.45	99.9
Normal	6.465*	

* Normal values are not obtainable in the literature. The value given here is from our series of normal dogs.

TABLE 5.—*Hypertrophy as Estimated by Method 1 B, C, D, E, F*

Ratio*	Group	Value of Ratio			Percentage of Normal
Fixed and prepared tissue					
TV to BW	Inoculated	0.00786	σ	0.00068	124.0
	Control	0.00674		0.00079	106.0
	Normal	0.00635		0.00075	
LV to BW	Inoculated	0.00394	σ	0.000397	131.0
	Control	0.00345		0.00035	115.0
	Normal	0.00300		0.00028	
S to BW	Inoculated	0.00171	σ	0.000418	148.0
	Control	0.00165		0.000306	142.0
	Normal	0.00116		(Not reported)	
RV to BW	Inoculated	0.00235	σ	0.000238	111.0
	Control	0.00180		0.000183	85.0
	Normal	0.00212		0.00216	
Fresh tissue					
A to BW	Inoculated	0.00099	σ	0.00219	126.0
	Control	0.00087		0.000158	111.8
	Normal	0.00078		(Not reported)	

* TV = total ventricular weight (fixed and prepared tissue).
BW = body weight.
LV = left ventricular weight (fixed and prepared tissue).
RV = right ventricular weight (fixed and prepared tissue).
S = septal weight (fixed and prepared tissue).
A = auricular weight (fresh tissue).

Figure 1 shows the relative proportion of overlap between the frequency polygons for the inoculated and the control series. It is this factor that is responsible for the greatly increased accuracy of this method over the gross methods of expressing cardiac hypertrophy. The fibers from the septum were generally found to be slightly larger than those from the apex, a fact that may perhaps find its explanation in the observation that the ventricular walls of a majority of the hearts were definitely thinned to two thirds or one half of the normal thickness at the particular apical point selected for the specimens. The findings of Goldberger [7] are corroborated by this work with the exception of cell volume, which we are unable to determine, owing to the fact that the

present thought on cardiac muscle [15] considers it a syncytium, with the result that the length of the cardiac muscle fiber cannot be determined. The findings of Tangl [8] fail to find support in our results.

STATISTICAL COMPUTATIONS

Computations 1 A, B, C, D, E, F.—The major difference between the inoculated and the control group, when the cases are plotted against the ratios of total heart weight and of individual heart chamber weights

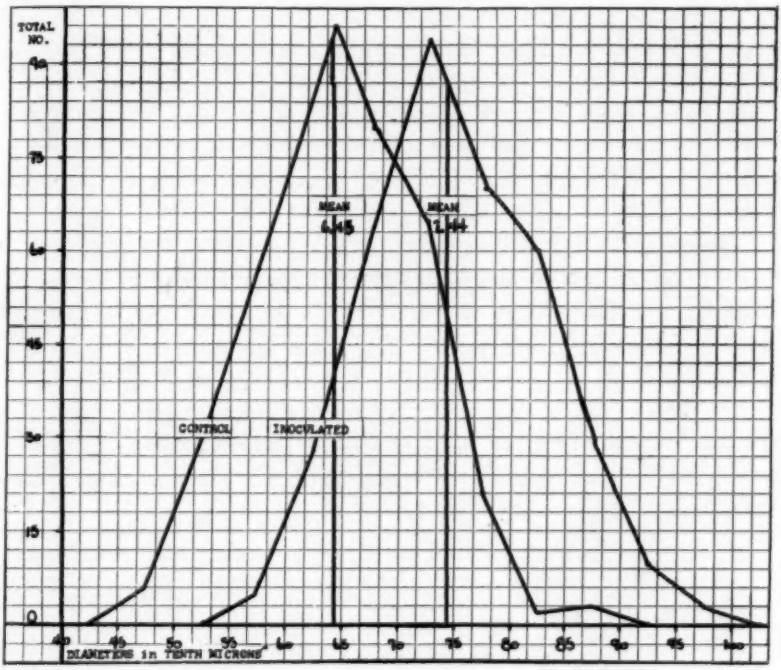

Fig. 1.—Individual muscle fiber diameters.

to body weight, consists of a skewness of the curves of the inoculated group to the higher ratio values, while the lower limits of the curves are roughly the same as those of the curves of the control group. This fact indicates that our ratios of cardiac hypertrophy based on body weight at death, and on the body area values derived therefrom, may perhaps be unduly influenced by the greater loss in body weight of the inoculated group. This would make such ratios, under our conditions, fail to express the degree of true cardiac hypertrophy actually present.

15. Strong, O. S., and Elwyn, A.: Bailey's Textbook of Histology, ed. 7, New York, William Wood & Company, 1925, p. 158.

Data on the heart weight at the beginning of the experiment are anatomically impossible to obtain, and even teleroentgenograms of dogs are inaccurate for the aforementioned estimations, with the result that we cannot, mathematically or statistically, estimate the relative effect of the greater loss in weight on these ratios. This matter will be further discussed under the head, Computation 2 Aa.

Computations 1 G, H, I, J.—The data relative to these computations have been previously discussed under the head, Estimations, Method 4 A, B, C.

Computations 2 Aa.—A moderate degree of negative [16] relationship between the ratio of fresh heart weight to body weight and the total number of minutes of exercise was found. The relationship is most marked in the inoculated group. This finding probably has its explanation in the observation that those dogs, dying of an intense toxemia, and therefore not living for the entire experimental period, as a general rule, showed the greatest ratios of heart weight to body weight.

Computation 2 Ab.—The ratio of heart weight to body weight and that of heart weight to body surface area show a positive correlation, indicating that these ratios express a number of common factors.

Computation 2Ac.—Control dogs show no apparent relationship between the ratio of heart weight to body weight, and body weight at death, while the inoculated series display a marked negative correlation. This fact suggests that the marked loss of body weight in the toxemic inoculated dogs may have had an undue influence on those dogs showing a remarkably high ratio of heart weight to body weight.

Computation 2 Ad.—A slight positive relationship was noted between the factors of the ratio of heart weight to body weight and gross fresh heart weight in grams.

Computation 2 Ae.—The average diameter of the muscle fibers, inasmuch as it is a single, definitely delimited measurement, is less subject to error than a ratio, in which a variation in either of its component factors affects the consequent ratio. The ratio of heart weight to body weight shows only a slight negative [16] relationship to the average muscle fiber diameter, and therefore we conclude that, at least under our particular experimental conditions, the ratio of heart weight to body weight is a less desirable method of expressing cardiac hypertrophy than the microscopic evidence afforded in increased diameter of the muscle fiber.

Computation 2 Ba.—In contrast to the findings in 2 Aa, we note that there is apparently no effect of prolonged exercise on the control

16. "Negative" refers to correlation in the sense that an increase in one of the factors of the correlation is in general accompanied by a decrease in the other correlated factor.

dogs, with the result that the average diameters of their muscle fibers remain at practically the same general level regardless of running time. A very definite moderate degree of positive relationship is noted between the length of exercise time and the average diameter of the muscle fibers in the inoculated series. This shows that those dogs that survived the initial toxemia and continued exercise under the effects of a prolonged constant toxemia responded with a greater increase in the average diameter of the muscle fibers than those dying earlier in the experimental series.

Computation 2 Bb.—The ratio of heart weight to body surface area, when plotted against the total number of minutes of exercise, shows a scatter so great as to indicate practically no relationship.

Computations 2 Ca, 2 Cb.—A high degree of positive relationship is noted between the ratio of heart weight to body weight and its component factors in the control series. The inoculated group shows the same positive interrelationship to heart weight, but is variable when examined in comparison with body surface area in square meters.

Computation 2 Cc.—When the ratio of heart weight to surface area is considered in its relationship to the average diameter of the cardiac muscle cells of that particular animal, we find that the inoculated series shows a slight degree of positive relationship. This indicates that there is a smaller degree of common factors between the ratio of heart weight to body area and average diameter of muscle fibers than is shown between the ratio of heart weight to body weight and average diameter of muscle fibers. The control series displays a much more marked negative relationship. Under these circumstances, the slight positive relation shown by the inoculated group is probably a result of the chance combination of experimental variables, and therefore unreliable to use as a basis for conclusions.

Computations 4 A, B.—The Pearson product-moment coefficient of correlation between the total weight of fixed and prepared ventricles and body weight at death we find to be 0.76 ± 0.2 for inoculated and 0.90 ± 0.2 for control dogs. The same coefficients for the correlation between the weight of fixed and prepared left ventricle and body surface area are 0.81 ± 0.2 for inoculated dogs, 0.93 ± 0.2 for controls, and 0.91 ± 0.02 for normal dogs. Since in all cases the value r is practically 4 PE_r, it can be concluded that a definite positive relationship exists between these factors. The common factors in these measurements we find to be from 65 to 87 per cent of the possible total, and such percentages indicate a relatively high degree of elimination of avoidable experimental error in the entire series.

Computation 4 C.—The weight of the fixed and prepared left ventricles when compared with the weight of the fixed and prepared right

ventricles by means of a coefficient of correlation reveals an r of 0.68 ± 0.2 for inoculated dogs, 0.82 ± 0.2 for controls, and 0.95 ± 0.02 for normal dogs. These figures show a definitely positive relationship, but of less degree than that shown by the ratios based on body weights. The common factors are estimated at a minimum of 49 per cent.

Computation 4 D.—The ratios of heart weight to body weight and the percentages of original body weight lost during the experimental series have been made the variable factors in a Pearson product-moment coefficient of correlation in order to determine whether the greater loss in body weight in inoculated dogs is responsible for the resultant ratios of heart weight to body weight. The following coefficients of correlation express the findings:

r Inoculated.....	0.50 ± 0.15	25 per cent common factors
r Control.....	0.31 ± 0.18	15 per cent common factors

An interpretation of these figures indicates that there is a slight positive correlation between the ratio of heart weight to body weight and the percentage of body weight lost, greater in the inoculated than in the control series. The uncommon factors are markedly predominant in these variables, and are in such excess that the common values bear little significant relationship to each other. Further calculations by Dr. Frank L. Griffin, professor of mathematics, Reed College, and by ourselves show that the increase in the ratio of heart weight to body weight can be more than accounted for by the greater percentage of body weight lost in the inoculated dogs. These calculations may be summarized as in table 6.

We consider it evident from the figures in table 6 that the greater ratio of heart weight to body weight of the inoculated dogs was due either to an actual decrease in the cardiac musculature during the experimental period or to the preponderant loss in body weight during the same time. The former is at variance with clinical findings, and therefore we assume that the greater percentage of body weight lost in the inoculated dogs affects the ratio of heart weight to body weight in such a manner as to render it relatively unreliable as a criterion of cardiac hypertrophy under our particular experimental conditions. The same conclusion may be applied to the ratios of heart weight to body surface area, as they are directly derived from the body weights at death. These conclusions do not deny that the ratios have value as estimates of cardiac hypertrophy, but to us they seem less reliable than other investigated methods.

Computations 5 A, B, C, D.—The reliability coefficients as shown in table 7 are self-explanatory, but are subject to the same criticism as discussed under the head, Computation 4 D. These figures illustrate the fallacy of depending on rough statistical data without a careful analysis

as to their meaning. The reliability coefficients indicate that a great deal of confidence may be placed in the ratio of heart weight to body weight and other similar ratios. This conclusion is at variance with the previously given evidence. In view of this fact it appears that if the ratios based on body weight at death were not unduly influenced by the variable percentage loss in body weight, the resulting ratios would be highly significant. Under our particular experimental conditions, these same ratios cannot be accepted as indicating nearly so great a degree of chance that difference is greater than zero as that actually shown, although as we have previously pointed out, a slight positive relationship is present. The ratio of left ventricular to right ventricular

TABLE 6.—*Summary of Calculations Showing That the Increase in the Ratio of Heart Weight to Body Weight Can Be Accounted for by the Greater Percentage of Body Weight Lost in the Inoculated Dogs*

Group	Probability That Heart Weight Is		Combined Probability for Entire Group That Heart Weight Is	
	Increased	Decreased	Increased	Decreased
Inoculated	0.06066	0.93934	1.274×10^{-14}	1.524×10^{-2}
	0.16602	0.83398		
	0.00639	0.99361		
	0.04006	0.95994	Ratio of likelihood in favor of decrease for all as against increase for all	
	0.02500	0.97500		
	0.35569	0.64431		
	0.00071	0.99929		
	0.63683	0.36317	$\frac{1.524 \times 10^{-2}}{1.274 \times 10^{-14}} = 1$ trillion plus	
	0.84850	0.15150		
	0.03593	0.96407		
	0.28774	0.71266		
	0.14007	0.85993		
Control	0.16354	0.83646	1.560×10^{-5}	1.191×10^{-3}
	0.34458	0.65542		
	0.64803	0.35197		
	0.70884	0.29116	Ratio of likelihood in favor of decrease for all as against increase for all	
	0.77637	0.22363		
	0.53586	0.46414		
	0.00734	0.99266		
	0.10749	0.89251	$\frac{1.190 \times 10^{-3}}{1.560 \times 10^{-5}} = 763{,}000$	
	0.59095	0.40905		
	0.07214	0.92786		
	0.15150	0.84850		

weight is uninfluenced by these factors, and represents a more nearly true value of the amount of relationship actually present. A chance selection, with equal probability of being true or false, would in itself result in a 50/100 selection.

Computation 6.—A comparison of the average ratios of heart weight to body weight of our series of normal dogs and those reported by Herrmann [9] is as follows:

Normal Group	Ratio of Heart Weight to Body Weight
Ours	0.00776 σ 0.000276
Herrmann's	0.00798 σ 0.0001036

These figures, for a group of but seven dogs in comparison with the larger series of two hundred dogs, show that we have approximated the reported series in our group with a reasonable degree of accuracy.

On the basis of this check, we concluded that the normal values quoted by Herrmann [9] are a satisfactory series, and we therefore discontinued our series. The normal values quoted are from Herrmann unless there is a specific mention to the contrary.

Computation 7.—Column 7 in table 1 gives an estimation of the general running ability according to a four-unit graded system of recording. This was done in order that we might know whether the amounts

TABLE 7.—*Reliability Coefficients*

	Ratio of				
	Heart Weight to Body Weight	Total Weight Fixed and Prepared Ventricles to Body Weight	Left Ventricle Weight to Body Weight	Right Ventricle Weight to Body Weight	Left Ventricle Weight to Right Ventricle Weight
Inoculated					
Average	0.00883	0.00778	0.00391	0.00234	1.765
Probable error	0.000242	0.000458	0.000268	0.000160	1.21
Control					
Average	0.00792	0.00675	0.00345	0.00181	1.904
Probable error	0.000226	0.000532	0.000236	0.000123	1.34
Normal					
Average	0.00798	0.00635	0.00300	0.00212	1.461
Probable error	0.0000698	0.000505	0.000189	0.000146	0.214
Normal and control					
Diff *	0.00006	0.00040	0.00045	0.00031	0.503
PE diff †	0.000236	0.000225	0.000302	0.000191	1.36
Diff/PE diff ‡	0.254	1.78	1.49	1.62	0.37
Chances	57 to 100	88 to 100	84 to 100	86 to 100	60 to 100
Normal and inoculated					
Diff	0.00085	0.00143	0.00091	0.00022	0.304
PE diff	0.000253	0.000216	0.000328	0.000216	1.23
Diff/PE diff	3.36	6.62	2.86	1.02	0.247
Chances	99 to 100	99 to 100	97 to 100	75 to 100	57 to 100
Control and inoculated					
Diff	0.00091	0.00103	0.00046	0.00053	0.199
PE diff	0.000331	0.000222	0.000350	0.000202	1.81
Diff/PE diff	2.75	4.64	1.31	2.62	1.10
Chances	97 to 100	99 to 100	81 to 100	96 to 100	77 to 100

* Diff = numerical difference between averages.

† PE = probable error of averages. Limits within which, plus and minus from the average, 50 per cent of the variables are found.

† PE diff = a calculated value (see reference) representing the variability of the two compared units, and therefore the expected mathematical variability.

‡ Diff/PE diff = actual numerical difference between averages divided by the expected variability. It represents the amount of chance elements in the two variable series, zero representing all correlation being due to chance and infinity representing measurement of identical units.

of exercise represented by the figures for total exercise time in the two groups were approximately equivalent. Using 1 as the unit for the best running ability and 4 as that for the poorest, we find the average to be, inoculated, 2.54, and control, 2.25. These average values indicate that the groups are largely equivalent to each other in running ability, with a majority of the dogs running satisfactorily. The poorer running ability in the inoculated series, while relatively slight, has its most logical explanation in the toxicity of this group, with the consequent cardiac embarrassment during periods of exertion.

Computation 8.—Columns 21 and 22, table 1, represent an unsuccessful attempt to estimate the ratio of heart weight to body weight at the beginning of the experimental period by using (1) the animal's own fresh heart weight at death as a normal, on the assumption that of any single values this represents the best obtainable estimate of cardiac weight at the beginning of the period, and (2) an average normal heart weight as reported by Herrmann.[9] As no conclusions can be drawn from the resultant figures, we discontinued the consideration of these ratios.

COMMENT

The inoculated dogs were more or less depressed or toxic throughout the period of the experiment. They showed more fatigue than the non-

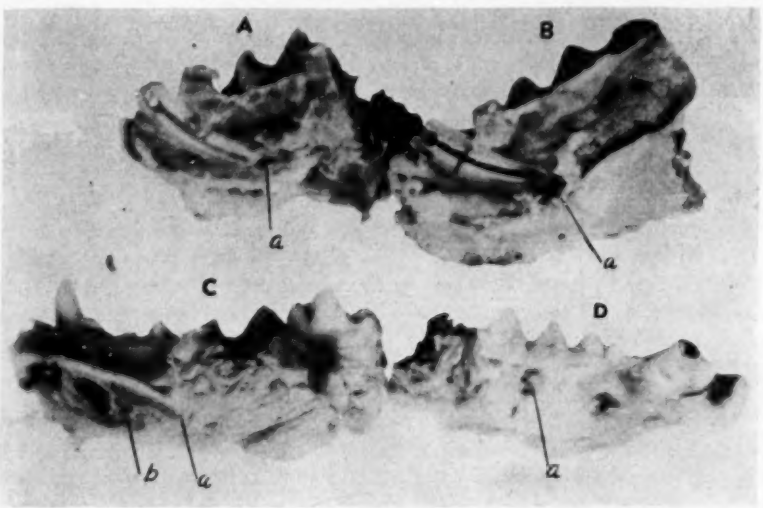

Fig. 2.—Gross jaw dissections from inoculated dogs: *A*, lower jaw of dog 16, showing an apical dental abscess (*a*); *B*, lower jaw of dog 1, showing an apical dental abscess (*a*); *C*, lower jaw of dog 17, showing an apical dental abscess (*a*) and a necrotic peridental membrane (*b*); *D*, lower jaw of dog 9, showing an apical dental abscess eroding the outer table of the jaw (*a*).

inoculated dogs during the periods of exercise. Artificial respiration was resorted to eight times in the case of the inoculated dogs, whereas at no time was it necessary with the control animals. Only 25 per cent of the inoculated dogs lived through the experimental time, in comparison with 83.5 per cent of the control series. Both deaths in the latter series resulted from fighting, while in the former group four dogs died from acute cardiac failure. The average loss in weight in the control series was 5.8 per cent, in comparison with 19.9 per cent among the inoculated dogs. There is, therefore, considerable clinical evidence, as

well, that the inoculated dogs were made chronically ill by reason of their dental infection. The average duration of life among them was one hundred and seventy-nine days, whereas in the control group it was two hundred and two days, and ten of these dogs were killed at the end of the experimental time. The six months' experimental period represents roughly one twentieth of the average lifetime of dogs, which in turn corresponds to about three years of a human lifetime. One cannot deny a certain suggestive similarity in clinical course and pathologic lesions between these inoculated dogs and human patients suffering from those myocardial changes that are believed to follow, at times, especially in the wake of chronic dental and chronic sinus infection.

Postmortem Observations.—The observations at autopsy in the inoculated series of dogs were briefly as follows:

Dog 1. Small dental abscess; very small vegetative mitral endocarditic lesion; no further lesions

Dog 2. Moderate dental abscess; small vegetative mitral and aortic endocarditic lesions; no further lesions

Dog 3. Small dental abscesses; moderate vegetative mitral and aortic endocarditic lesions; no further lesions

Dog 5. Large dental abscess; moderate mitral and aortic verrucose endocarditic lesions; bilateral hilar tuberculosis; terminal bronchopneumonia; acute cardiac dilatation

Dog 7. Small dental abscess; moderate mitral and aortic vegetative and verrucose endocarditic lesions; tapeworm; no further structural changes

Dog 10. Moderate dental abscess; very small mitral endocarditic lesions; no further lesions

Dog 16. Moderate dental abscess; moderate mitral and aortic verrucose endocarditic lesions; ulcerative colitis; acute cardiac dilatation

Dog. 22. Small dental abscess; moderate aortic verrucose endocarditic lesion; mange; tapeworm

Dog. 24. Small dental abscess; moderate verrucose and vegetative mitral and aortic endocarditic lesions; multiple abscesses in pectoralis majoris and minoris and rectus abdominis

Dog 8. Small dental abscess; small mitral endocarditic lesion; tapeworm

Dog 9. Moderate dental abscess with erosion of outer table of mandible; small mitral and aortic vegetative endocarditic lesion; no further structural changes

Dog 17. Moderate dental abscess; small mitral endocarditic lesion; terminal bronchopneumonia; acute cardiac dilatation

The observations at autopsy in the control series were as follows:

Dog 6. Emaciation

Dog 13. Emaciation

Dog 20. No gross structural changes

Dog 25. Terminal bronchopneumonia; death by fighting

Dog 26. Mange; tapeworm

Dog 27. Death by fighting; evisceration; tapeworm

Dog 11. Moderately thin

Dog 14. No gross structural changes
Dog 28. No gross structural changes
Dog 29. No gross structural changes
Dog 30. No gross structural changes
Dog 31. No gross structural changes
Dog 12. Control dog with endocarditis. No dental abscesses; moderate vegetative aortic and mitral endocarditic lesions; ulcerative colitis; no demonstrable primary focus of infection noted

Dog 12 was carried in the control series until autopsy was performed. No dental abscesses were seen, and no primary focus of infection could be identified at necropsy. The causative organism was unknown. This dog showed signs of toxicity during its life. The ratio of heart weight to body weight showed an apparent cardiac hypertrophy with a value of 0.00965. The average diameter of the apical cardiac muscle fibers of this dog was 7.31 microns. It was not included in either of our series on account of the unknown nature of the causative organism.

The histologic examination of the myocardium of the inoculated animals showed from a mild to a moderate grade of patchy parenchymatous degeneration of the individual fibers, which were observably increased in transverse diameter. Individual cell nuclei were found that showed irregularity, pyknosis, a deeper concentration of the chromatin and in some cases a partial or a complete doubling. A slight diffuse round cell infiltration by lymphocytes was seen in the majority of the sections. The cells were located for the most part perivascularly. Plasma cells were occasionally seen. A slight degree of fatty change, most marked subendocardially, was noted in dog 17. Multiple small focal hemorrhages were noted in the myocardium of dog 3. No abnormal changes were seen in the myocardium in any of the control or normal dogs. The heart muscle of dog 12 was accidentally not examined.

Many sections were stained and examined for micro-organisms within the tissue, but no organisms could be found. There are, seemingly, three possible explanations for the myocardial changes. The patchy areas of degeneration may have been produced by micro-organisms still present in the areas, but not recognized. The micro-organisms, on the other hand, may have been present in the tissues and been destroyed by the antibacterial properties of the heart muscle. Or, again, the myocardial changes may have been the result of toxins alone, and the blood stream have been sterile at all times. Blood cultures were made three times from inoculated dogs, but always were sterile.[17]

Reliability of Results.—Although the experimental groups were limited to twelve dogs in each series, in order that the experiment might not be too cumbersome and unwieldy, the results received therefrom appear

17. Dr. E. C. Rosenow prepared and studied sections from blocks of these tissues and corroborated these findings.

to be relatively reliable. The experimental error is estimated to be not over 1.5 per cent on an average. The methods have apparently measured from 49 to 87 per cent of the possible factors concerned in the problem, as noted under the heading, Computation 4 A, B. Thus the results can be considered to indicate that increasing experimental errors have been avoided and the unavoidable reduced to a minimum. It appears, therefore, that our twenty-four experimental animals comprise a population sufficiently large for the purpose of this study.

SUMMARY

Dental abscesses were demonstrated in all inoculated dogs. There were no other constant extracardiac structural changes.

The hearts of inoculated dogs constantly showed very small vegetative or verrucose mitral and (or) aortic endocarditic lesions, patchy parenchymatous degeneration, nuclear changes, increase in the diameter of the muscle cell, and a slight round cell infiltration.

A positive relationship between experimentally produced focal dental infection and cardiac hypertrophy as measured by diameters of muscle cells was noted.

Direct measurement of diameters of cardiac muscle fibers, under our experimental conditions, is a more reliable criterion of cardiac hypertrophy than ratios of heart weight to body weight or of heart weight to body surface area.

Stress and strain in the absence of focal infection did not affect the gross or the microscopic characteristics of the heart.

EXPERIMENTAL EDEMA AND LIPEMIA PRODUCED BY REPEATED BLEEDING*

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In 1928, Leiter [1] produced edema in dogs by bleeding them, separating the plasma from the red blood cells, making up the original volume of blood with Locke's solution and then reinjecting the resulting suspension of blood cells (plasmapheresis). The result was a striking depletion of plasma protein. His impression at the time was that the edema was associated with a low plasma protein, 3 per cent or less.

Barker and Kirk [2] were able to produce edema in dogs by removing the plasma proteins in essentially the same manner. It was their impression that edema was associated with a low plasma protein, 0.8 per cent or less. The kidneys of their dogs showed some tubular destruction, plasma cell and round cell infiltration and some increase in interstitial connective tissue. There was a moderate accumulation of fat in the tubules. Some glomeruli showed atrophy and occasional hyalinization of Bowman's capsule. These workers considered all these renal changes secondary to the prolonged hypoproteinemia. The urine from time to time showed albumin and casts. The plasma cholesterol level was rather irregular and at times moderately increased. The blood volume remained about the same during the period of edema. They concluded that the experimental disease in dogs might be the same as human lipoid nephrosis.

In a complete summary of his work on experimental edema in dogs secondary to plasmapheresis, Leiter [3] again stated that the edema was due to a low concentration of plasma protein, 3 per cent or less. He laid no particular stress on the concentration of plasma albumin. The cholesterol level was irregular, but never greatly or consistently elevated. The kidneys of his dogs showed infarcts and localized areas of suppuration in the cortex. These the author explained as being secondary to emboli originating in the heart and due to lack of asepsis during the punctures of the heart. He also encountered the lesions described by Barker and Kirk and considered them as cases of spontaneous contracted kidneys in the dogs. All the edema fluids were low in protein

* Submitted for publication, Sept. 22, 1931.

1. Leiter, L.: Proc. Soc. Exper. Biol. & Med. **26**:173, 1928.
2. Barker, H. M., and Kirk, E. J.: Arch. Int. Med. **45**:319, 1930.
3. Leiter, L.: Arch. Int. Med. **48**:1, 1931.

(from 0.02 to 0.47 per cent). Leiter concluded that while the edema was doubtless of the nephrotic type, the experimentally induced state in the dog was not nephrosis.

Shelburne and Egloff [4] were able to produce edema in one dog by giving it a diet low in protein but adequate in carbohydrate, fat and water. The chemical, pathologic and clinical observations in this dog were essentially the same as those in two other dogs in which the edema was produced by plasmapheresis. On the whole, their results were in agreement with those of Barker and Kirk; the results differed in that the kidneys of their dogs showed only an accumulation of fat in the tubules; the urine also was free from albumin and casts. They were able to demonstrate glomerular lesions of various kinds in so-called normal dogs. In addition, they showed that with a given low concentration of plasma protein the amount of edema could be increased by giving sodium chloride and sodium bicarbonate. The edema fluids in their dogs were low in protein. They agreed with Leiter that the experimental disease in dogs is not nephrosis.

The purpose of my experiments was to determine whether edema could be produced simply by bleeding an animal repeatedly over a prolonged period. During the course of the work, the changes in the plasma proteins and lipoids were noted. The kidneys of all the animals and the livers of two were likewise analyzed for their lipoid content.

EXPERIMENTAL PROCEDURE

About thirty well nourished adult male rabbits were selected and kept under observation for at least one month before any work was undertaken. The fifteen rabbits finally selected showed hemoglobin values over 100 per cent and red cell counts over 7,000,000. The white cell count never exceeded 8,000. The urine was normal on repeated examinations. The diet consisted of carrots, parsnips, alfalfa hay, corn and water.

The work was carried out at an altitude of approximately 4,000 feet (1,219.2 meters) above sea level and at a barometric pressure of about 640 mm. of mercury.

The rabbits were bled at intervals of from 36 to 45 hours; from 40 to 75 cc. of blood was removed each time from the right ventricle of the heart. This was done under local anesthesia, with a long, thin needle.

The blood volume was determined by the method of Keith, Rowntree and Geraghty.[5]

The plasma proteins were fractionated and precipitated by the method of Howe.[6] The total protein nitrogen and the albumin nitrogen were determined by the micro-Kjeldahl technic of Berglund.[7] The procedure of Folin and Wu [8] was used to calculate the nonprotein nitrogen.

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4. Shelburne, S. A., and Egloff, W. C.: Arch. Int. Med. **48**:51, 1931.
5. Keith, N. M.; Rowntree, L. G., and Geraghty, J. T.: Arch. Int. Med. **16**:547, 1915.
6. Howe, P. E.: J. Biol. Chem. **49**:109, 1921; **57**:235, 1923.
7. Berglund, H.: Personal communication.
8. Folin, O., and Wu, H.: J. Biol. Chem. **38**:81, 1919.

TABLE 1.—*Rabbit 1*

Date	Amount of Blood Taken, Cc.	Hemoglobin	Erythrocytes	Leukocytes	Urine	Total Blood Volume, Cc.	Plasma Volume, Cc.	Plasma Fats, Mg. per 100 Cc.			Total Plasma Protein, per Cent	Plasma Albumin, per Cent	Plasma Globulin, per Cent	Non-protein Nitrogen, Mg. per 100 Cc.	Wt., Lb.	Comment
								Saponifiable	Nonsaponifiable	Total						
1/ 5/31	50	105	7,650,000	7,200	—	150	121	663	82	720	7.86	6.80	1.06	47	8	
1/ 8/31	40	...	.....	.....	—	...	...	756	80	836	8.06	4.73	3.33	26	8	
1/10/31	40	...	.....	.....	—	...	...	801	89	890	7.20	5.24	1.96	48	...	
1/13/31	30	...	.....	.....	Alb. +	146	119	988	84	1,072	5.05	2.94	2.11	..	7¾	
1/16/31	50	70	4,200,000	14,000	Alb. +	...	...	516	96	912	....	....	....	..	...	
1/19/31	35	...	.....	.....	—	...	...	672	104	776	9.28	....	....	30	7¾	
1/21/31	50	...	.....	.....	—	140	110	640	201	851	....	2.84	....	37	...	
1/22/31	40	65	3,920,000	13,400	Alb. +	...	...	1,140	410	1,550	5.10	2.57	2.53	34	7¾	Slight edema of scrotum
1/25/31	40	...	.....	.....	—	...	...	1,290	302	1,610	5.16	3.12	2.04	31	...	Edema of scrotum ++; bleeding discontinued
2/ 3/31	50	85	5,888,000	8,000	—	...	152	682	102	704	4.51	....	....	38	8	No edema
2/ 4/31	50	...	.....	.....	Alb. +	...	...	514	274	788	4.96	2.58	2.38	27	8	No edema
2/ 6/31	50	60	.....	.....	—	...	...	666	144	780	4.56	....	....	40	7¾	No edema
2/ 8/31	50	...	.....	.....	Alb. +	110	85	1,752	512	2,263	7.67	2.66	5.01	44	7	No edema
2/10/31	45	45	3,744,000	7,400	—	...	...	263	64	327	5.15	2.57	2.58	35	7	No edema
2/12/31	45	45	3,464,000	10,000	Alb. +	...	...	2,094	108	2,202	5.74	2.39	3.35	38	7¼	Slight edema of scrotum
2/14/31	50	39	3,200,000	7,000	—	...	...	.....	...	1,246	5.48	2.13	3.35	71	7¼	Edema of scrotum ++
2/16/31	60	30	.....	.....	Alb. +	102	80	.....	...	1,950	5.20	2.29	2.91	89	7¼	Edema of scrotum +++
2/18/31	65	20	1,680,000	5,600	Alb. +, R.B.C., W.B.C., casts	...	...	1,404	79	1,483	5.09	1.87	3.22	93	7½	Edema of scrotum and anterior abdominal wall ++++; edema of hind legs ++; ascites

An ether-alcohol-ether extract was used in the analyses of the plasma lipoids. Ten cubic centimeters of plasma was dried and then extracted for three hours with warm ether. This was followed by extraction for one hour with boiling alcohol. The sample was then extracted for one hour with warm ether. The combined extracts were dried to a constant weight in a vacuum oven.

The total extracts were then separated into saponifiable (neutral fat) and nonsaponifiable (sterol) fractions. The saponification was carried out by boiling the sample under a reflex condenser with alcoholic potassium hydroxide for one hour. The alcoholic potassium hydroxide was then evaporated on a steam bath and the resulting residue extracted for twelve hours with cold ether. This ether extract was designated as the unsaponifiable or sterol fraction.

Tissues (kidney and liver) and samples of urine were subjected to exactly the same procedure in the determination of their total lipoid, saponifiable and nonsaponifiable contents.

RESULTS

Rabbit 1 (table 1).—In rabbit 1, an anemia and a milky plasma with a true increase in fat developed after the third bleeding. The plasma continued to be milky, but the actual lipoid content on one occasion fell to nearly the normal level. The plasma lipoid level rose sharply just when the animal showed signs of edema.

A slight edema of the scrotum was noted on the seventeenth day. This rapidly increased within the following four days. The concentration of the total plasma protein fell slightly (5.10 per cent), while the concentrations of the albumin and globulin were about equal. On the twentieth day, bleeding was discontinued for one week. The edema and the lipemia rapidly subsided, and the plasma became clear.

Bleeding was then resumed, and the edema of the scrotum promptly returned on the fifth day. The plasma again became milky, and the total plasma lipoid was greatly increased. The rise was always most marked in the neutral fat fraction. The concentration of the total plasma protein was practically the same as before (5.74 per cent), but that of the albumin had dropped to 2.39 per cent and that of the globulin had increased to 3.35 per cent. The albumin-globulin ratio continued to be reversed throughout the remaining lifetime of the animal.

The edema soon extended to the anterior abdominal wall, and at the same time ascites could be demonstrated. The animal now walked with difficulty. On the day before death there was a moderate edema of the hind legs. The plasma lipoid level continued to be high, but the milky appearance disappeared several days before death. The animal lost about a pound in weight and became rather emaciated in spite of the massive edema of some of its tissues. There was a marked secondary anemia at all times. The total blood volume and the plasma volume fell as the bleeding progressed. The total nonprotein nitrogen (plasma) began to increase about four days before death and finally rose to 93 mg. per hundred cubic centimeters.

Fat could always be found in the urine after the lipemia was well established. It showed practically the same saponifiable and nonsaponifiable values as did the plasma fat. A small amount of albumin appeared in the urine from time to time. Numerous red blood cells, white blood cells and some granular casts were found in the urine on the day preceding the animal's death.

A summary of the data shows that the rabbit survived for forty-six days and lost 810 cc. of blood. This represents an approximate loss of 29 Gm. of albumin and 22 Gm. of globulin. The total amount of plasma protein lost amounted to 51 Gm.

Postmortem Examination: The following observations were made: marked edema of scrotum and anterior abdominal wall; massive ascites; moderate hydrothorax; no hydrocele; moderate subcutaneous edema of hind legs; moderate fatty metamorphosis of liver; surface of kidneys smooth and golden yellow; renal medulla pale; renal cortex golden yellow and all markings lost; remaining viscera pale; only trace of blood in large vessels; bone marrow yellow; great decrease in subcutaneous, mesenteric and perirenal fat.

Microscopic Examination: Sections of the kidney stained with hematoxylin-eosin were normal. Fresh frozen sections stained with sudan III showed some overstaining of the cytoplasm of the convoluted tubules, but no fat droplets. Fresh frozen sections of the liver, stained with sudan III, showed the cytoplasm of the cords moderately filled with fat droplets.

Rabbit 2 (table 2).—In this animal a secondary anemia and lipemia were slow in developing. The increase in plasma fat was not nearly so marked as in rabbit 1, although the plasma was at times quite milky. The rise was always most marked in the neutral fat fraction. The concentration of the total plasma protein fell very little. The concentration of albumin was, however, moderately decreased, while that of globulin was increased. There was no rise in nonprotein nitrogen. The total blood volume and the plasma volume were decreased as the bleeding was continued.

After the lipemia was well established, the urine showed a moderate amount of fat. The saponifiable and nonsaponifiable values were about the same as those found in the plasma. At times there was a small amount of albumin in the urine.

The animal never showed edema. As the bleeding progressed, the anemia became very marked, there was a great loss of weight, and the rabbit became very emaciated. There was a watery diarrhea for several days before death.

A summary of the data shows that the animal survived the bleeding for thirty-five days and lost 782 cc. of blood. During this time,

TABLE 2.—*Rabbit 2*

Date	Amount of Blood Taken, Cc.	Hemoglobin	Erythrocytes	Leukocytes	Urine	Total Blood Volume, Cc.	Plasma Volume, Cc.	Plasma Fats, Mg. per 100 Cc.			Total Plasma Protein, per Cent	Plasma Albumin, per Cent	Plasma Globulin, per Cent	Non-protein Nitrogen, Mg. per 100 Cc.	Wt., Lb.	Comment
								Saponifiable	Nonsaponifiable	Total						
1/13/31	37	107	7,200,000	7,200	—	150	121	698	78	776	7.20	5.76	1.44	38	7½	
1/16/31	50	...	.....	.....	—	...	...	698	93	791	7.02	4.96	2.05	40	...	
1/19/31	50	...	.....	.....	—	...	...	514	360	874	8.89	....	....	39	7	
1/21/31	30	...	.....	.....	Alb. +	141	114	781	120	901	6.03	2.42	3.61	34	...	
1/23/31	45	85	.....	.....	—	...	...	721	118	893	5.43	2.29	3.14	49	7	
2/ 2/31	50	80	3,720,000	8,600	Alb. +	141	120	722	134	856	9.65	2.56	7.09	51	7	
2/ 4/31	50	70	.....	.....	—	...	...	803	109	1,012	9.17	2.76	6.41	32	...	
2/ 6/31	50	65	.....	.....	—	...	...	1,010	81	1,091	5.78	....	....	36	6½	
2/ 8/31	50	50	.....	.....	—	...	....	1,256	76	1,332	5.76	2.99	2.76	35	6½	
2/10/31	50	40	2,200,000	9,600	Alb. +	...	...	1,376	100	1,476	6.61	3.17	3.44	45	6	
2/11/31	50	30	1,232,000	21,000	—	130	108	928	76	1,004	7.60	2.36	5.24	34	6	Listless, no appetite
2/12/31	30	28	1,200,000	15,000	Alb. +	...	...	755	205	960	8.13	....	....	39	5½	Listless, no appetite
2/14/31	50	30	.....	.....	Alb. +	...	...	706	112	818	6.88	2.56	4.32	35	5½	Emaciated
2/15/31	50	30	740,000	21,000	Alb. +	...	...	742	72	814	5.97	2.08	3.89	32	5	Emaciated
2/16/31	65	36	.....	.....	Alb. +	100	86	1,230	84	1,314	7.69	....	....	40	5	Greatly emaciated
2/17/31	75	25	832,000	15,000	Alb. +	...	...	1,537	155	1,692	6.80	2.26	4.54	39	4¾	Watery diarrhea

approximately 23 Gm. of albumin and 32 Gm. of globulin were removed. The total amount of plasma protein lost amounted to 55 Gm.

Postmortem Examination: The observations were as follows: great emaciation; no edema; practically no subcutaneous mesenteric or perirenal fat; all tissues pale; moderate fatty metamorphosis of liver; surfaces of kidneys smooth; renal medullae pale and cortices golden yellow; all markings obliterated; bone marrow yellow, except at extreme ends.

Microscopic Examination: Sections of the kidneys stained with hematoxylin-eosin were normal. Fresh frozen sections stained with sudan III showed some overstaining of the cytoplasm of the convoluted tubules, but no fat droplets. Fresh frozen sections of the liver, stained with sudan III, showed the cytoplasm of the cords moderately filled with fat droplets.

Rabbit 3 (table 3).—This animal was the most resistant one of the group. A secondary anemia developed rapidly, but never became as severe as in rabbits 1 and 2, even though more blood was lost. The lipemia was slow in developing, but once it was established it became the most marked of any. During the last twenty-one days of the animal's life, the total plasma fat was generally over 2,000 mg., and at one time it rose to over 4,800 mg. per hundred cubic centimeters. This striking increase was always most marked in the neutral fat fraction. The concentration of the total plasma protein showed little reduction. At times it rose to over 8 and 9 per cent. The concentration of albumin fell rapidly and at one time was only 1.61 per cent, the lowest concentration encountered in any one of the four rabbits. The globulin concentration was simultaneously increased. The plasma nonprotein nitrogen was never increased. The total blood volume and the plasma volume were decreased as the bleeding progressed.

The urine began showing large amounts of fat several days after the onset of the lipemia. This was mostly neutral fat. At times there was a small amount of albumin, and on one occasion, a few granular casts

The rabbit never became edematous. There was considerable loss in weight, and the animal became listless and greatly emaciated. There was a watery diarrhea for about one week before death.

A summary of the data shows that this rabbit lost 2,105 cc. of blood and lived seventy-four days. During this time 55 Gm. of albumin and 93 Gm. of globulin were removed. The total loss in plasma protein was 148 Gm.

Postmortem Examination: Autopsy showed: great emaciation; tissues tough and leathery; no edema; no subcutaneous, mesenteric or perirenal fat; liver pale, without gross fatty metamorphosis; kidneys smooth

TABLE 3.—*Rabbit 3*

Date	Amount of Blood Taken, Cc.	Hemoglobin	Erythrocytes	Leukocytes	Urine	Total Blood Volume, Cc.	Plasma Volume, Cc.	Plasma Fats, Mg. per 100 Cc.			Total Plasma Protein, per Cent	Plasma Albumin, per Cent	Plasma Globulin, per Cent	Non-protein Nitrogen, Mg. per 100 Cc.	Wt., Lb.	Comment
								Saponifiable	Nonsaponifiable	Total						
2/ 3/31	50	110	7,474,000	6,800	—	149	119	222	126	348	9.24	6.56	2.68	35	7¼	
2/ 5/31	50	..	.....	.....	—	...	...	310	91	401	6.40	3.39	3.11	36	...	
2/ 7/31	50	..	.....	.....	—	...	...	334	80	414	6.58	3.38	3.20	36	...	
2/ 9/31	50	..	.....	.....	Alb. +	...	...	436	95	521	5.43	4.38	1.05	30	...	
2/11/31	50	58	3,100,000	5,000	Alb. +	182	124	488	129	617	4.82	3.08	1.74	41	7¼	
2/14/31	50	..	.....	.....	Alb. +	...	...	512	111	623	6.82	2.89	3.98	32	...	
2/16/31	50	50	.....	.....	Alb. +	...	...	540	80	620	7.49	2.76	5.73	30	...	
2/18/31	50	40	1,984,000	9,600	—	155	110	319	82	401	6.48	2.76	4.70	32	7	
2/22/31	60	50	.....	.....	Alb. +	...	...	344	89	433	6.80	2.73	4.07	29	...	
2/24/31	55	50	2,976,000	6,600	—	...	...	387	91	478	6.93	2.54	4.39	34	7¼	
2/26/31	60	45	.....	.....	Alb. +	...	...	535	84	519	6.25	1.83	4.42	34	...	
2/28/31	60	40	.....	.....	Alb. +	...	...	597	93	690	9.14	2.61	6.53	35	...	
3/ 2/31	45	35	2,040,000	10,600	—	119	102	757	99	856	8.61	1.78	6.83	47	...	
3/ 4/31	45	28	.....	.....	Alb. +	...	...	1,251	169	1,420	9.25	2.00	7.25	21	7	
3/ 6/31	45	25	.....	.....	Alb. +	...	...	1,359	201	1,560	8.88	1.61	7.27	34	...	
3/ 9/31	55	35	.....	.....	—	...	...	911	102	1,031	7.25	2.26	4.99	46	6½	
3/11/31	45	45	2,720,000	7,600	Alb. +, R.B.C., W.B.C., casts	145	120	856	83	939	6.28	2.28	4.00	43	6½	
3/14/31	50	50	.....	.....	Alb. +	...	...	937	89	1,026	6.75	2.23	4.52	44	6½	
3/16/31	60	35	3,440,000	27,800	Alb. +	...	...	1,193	98	1,291	6.44	2.25	4.19	45	6½	
3/18/31	55	30	.....	.....	—	...	...	779	150	929	6.52	2.93	3.59	49	6½	
3/21/31	50	35	.....	.....	—	157	113	678	169	847	6.41	....	....	43	...	
3/23/31	60	30	.....	.....	—	...	...	1,499	291	1,790	6.78	2.60	4.18	66	6½	
3/25/31	60	35	.....	.....	Alb. +	...	...	1,519	301	1,920	8.29	....	....	30	...	
3/27/31	45	30	3,500,000	15,200	Alb. +	...	...	1,735	288	2,023	7.25	3.30	4.95	33	6¾	Listless, poor appetite
3/29/31	60	32	.....	.....	—	...	...	1,944	356	2,300	7.93	2.81	5.11	45	7	Listless, poor appetite
3/30/31	60	28	.....	.....	—	...	...	2,726	204	2,930	7.68	2.50	5.10	43	...	Listless, poor appetite
4/ 1/31	60	25	2,400,000	12,600	Alb. +	128	102	2,667	214	2,881	....	....	....	..	6¼	Listless, poor appetite
4/ 2/31	50	20	.....	.....	—	...	...	2,792	271	3,063	7.67	2.13	5.54	24	6	
4/ 4/31	65	28	.....	.....	—	...	...	2,022	174	2,169	8.23	2.50	5.73	20	6¼	
4/ 5/31	60	25	.....	.....	—	...	...	1,622	162	1,784	7.62	2.29	5.23	30	...	
4/ 7/31	60	30	.....	.....	—	...	...	2,084	502	3,486	7.96	2.93	5.03	41	6	Very listless
4/ 8/31	60	28	.....	.....	—	...	...	3,401	273	3,674	8.37	2.14	6.23	20	6	
4/ 9/31	60	25	.....	.....	—	...	...	.....	...	.....	6.63	2.00	4.63	40	6	
4/10/31	60	25	2,160,000	8,600	—	104	87	998	97	1,095	6.80	2.08	4.73	36	6	
4/12/31	60	20	.....	.....	—	...	...	.....	...	3,355	7.01	2.75	4.26	55	5½	Emaciated, watery diarrhea
4/14/31	60	30	.....	.....	—	...	...	4,549	301	4,850	6.98	2.81	4.17	40	5	Emaciated, watery diarrhea
4/17/31	70	30	.....	.....	—	...	...	1,903	297	2,200	6.70	2.01	4.69	31	5	Emaciated, watery diarrhea
4/18/31	70	30	2,296,000	12,700	—	...	...	2,193	271	2,464	6.07	2.21	4.86	41	5	Emaciated, watery diarrhea

and pale; renal cortex and medulla both pale; remaining tissues very pale; practically no blood in heart and large vessels; bone marrow very red throughout.

Microscopic Examination: Sections of the kidneys stained with hematoxylin-eosin were normal. Fresh frozen sections stained with sudan III failed to show fat of any kind. Sections of the liver stained with hematoxylin-eosin showed some destruction of the cords. Sections stained with sudan III did not show fat.

Rabbit 4 (table 4).—A severe anemia developed rapidly, and after the second bleeding the plasma was distinctly milky. There was a true increase in plasma fat, which persisted throughout the lifetime of the animal. This increase was most pronounced in the neutral fat fraction. The milky appearance of the plasma was lost about four days before the rabbit died; nevertheless the amount of extractable fat remained greatly increased.

A slight edema of the scrotum was noted on the ninth day after bleeding was started. The plasma lipoids at the time were 1,603 mg. per hundred cubic centimeters; the total plasma protein was 6.37 per cent; the albumin, 2.53 per cent, and the globulin, 3.84 per cent. There was no striking deviation from these values throughout the remainder of the experiment. On the last analysis, the albumin fell to 1.88 per cent. The edema rapidly increased and soon involved the greater part of the anterior abdominal wall. Massive ascites was demonstrable on the twentieth day, and the animal now had difficulty in walking.

After the twelfth day, the plasma nonprotein nitrogen began to increase and on the day of death rose to 144 mg. per hundred cubic centimeters. The blood and plasma volumes fell as the bleeding progressed.

The urine began to show large quantities of fat soon after the lipemia was well established. This was chiefly neutral fat. The urine was albumin-free for the most part. Three of the analyses showed a small amount.

In spite of the marked subcutaneous edema and ascites, the rabbit lost weight and became emaciated. A watery diarrhea developed a few days before the animal's death.

A summary of the data shows that the rabbit survived twenty-four days with a blood loss of 615 cc. Eighteen grams of albumin and 21 of globulin were removed. The total amount of plasma protein lost was 39 Gm.

Postmortem Examination: The observations were: great emaciation; marked decrease in subcutaneous, mesenteric and perirenal fat; scrotum four times normal size; subcutaneous tissue of scrotum and anterior abdominal wall glassy, gelatinous and about 2 cm. thick; no hydrocele; about 30 cc. of clear, straw-colored fluid in peritoneal cavity;

TABLE 4.—*Rabbit 4*

Date	Amount of Blood Taken, Cc.	Hemoglobin	Erythrocytes	Leukocytes	Urine	Total Blood Volume, Cc.	Plasma Volume, Cc.	Plasma Fats, Mg. per 100 Cc.			Total Plasma Protein, per Cent	Plasma Albumin, per Cent	Plasma Globulin, per Cent	Non-protein Nitrogen, Mg. per 100 Cc.	Wt., Lb.	Comment
								Saponifiable	Nonsaponifiable	Total						
3/ 9/31	50	105	7,040,000	7,200	—	164	100	702	72	772	8.17	4.93	3.24	41	6½	
3/12/31	40	60	.....	.....	—	...	...	542	16	558	7.14	5.32	2.09	45	6½	
3/14/21	50	55	.....	.....	—	...	...	876	106	1,082	6.60	2.83	3.77	54	6	
3/16/31	45	45	.....	.....	—	90	65	1,156	56	1,212	7.01	2.34	4.67	47	6	
3/18/31	55	30	.....	.....	—	...	...	1,502	102	1,603	6.37	2.53	3.84	45	5¾	Slight edema of scrotum
3/21/31	50	25	800,000	14,000	—	...	...	1,898	674	2,572	7.21	2.34	4.87	45	5½	Increasing edema of scrotum
3/23/31	60	25	.....	.....	—	...	...	919	183	1,102	6.06	3.48	2.58	60	5½	Edema of scrotum +++
3/25/31	60	25	.....	.....	Alb. +	64	53	1,458	144	1,602	5.37	2.60	2.79	76	5½	Edema of scrotum +++
3/27/31	45	25	1,520,000	10,000	—	...	...	1,142	64	1,206	5.35	2.57	2.78	80	5¼	Edema of scrotum ++++
3/29/31	60	22	.....	.....	Alb. +	...	...	1,425	126	1,551	5.77	2.39	3.38	89	5¼	Edema of scrotum and anterior abdominal wall ++++
3/31/31	45	20	1,840,000	13,000	—	...	...	508	736	1,244	5.81	2.81	3.47	95	5	Ascites
4/ 2/31	55	15	1,120,000	12,000	Alb. +	...	...	1,309	235	1,544	6.36	1.88	4.48	144	5	Edema of scrotum and anterior abdominal wall ++++, ascites, watery diarrhea

bilateral hydrothorax; marked fatty metamorphosis of the liver; some fat in spleen; kidneys smooth and pale; renal surfaces golden yellow; renal cortices golden yellow; renal medullae very pale and all markings lost; bone marrow yellow except at extreme ends.

Microscopic Examination: Sections of kidneys stained with hematoxylin-eosin showed a moderate destruction of the convoluted tubules with some round cell infiltration. There was a slight increase in interstitial tissue. The glomeruli were normal. Fresh frozen sections stained with sudan III showed some overstaining of the cytoplasm of the convoluted tubules. There were no fat droplets. Sections of the liver stained with hematoxylin-eosin showed moderate vacuolization of the cytoplasm of the cords. Fresh frozen sections stained with sudan III showed a moderate amount of fat.

SUMMARY OF RESULTS

Of the original fifteen rabbits, only four lived long enough to be of value. All were subjected to exactly the same procedure. In two of the animals (nos. 1 and 4) there developed edema of the scrotum and anterior abdominal wall, massive ascites and hydrothorax. Rabbit 1 also had moderate edema of the hind legs. The edema rapidly subsided if the bleeding was discontinued (rabbit 1), but soon returned when the bleeding was resumed. The remaining two never showed a trace of edema.

All the rabbits were bled until they died. The average duration of life from the beginning of the bleeding was about one month. Rabbit 3 was unusual in that it survived nearly two and one-half months. The total amount of blood removed ranged from 615 cc. (rabbit 4) to 2,105 cc. (rabbit 3). The amount of plasma protein actually lost ranged from 39 to 148 Gm. This is considerably more than is indicated by the protein concentrations expressed in percentages. All this is, however, consistent when the great decrease in blood volume and plasma volume is considered.

After several bleedings, all the animals presented a lipemia. The time of onset of the lipemia varied considerably in the different rabbits. Lipemia secondary to repeated hemorrhage in rabbits was first reported by Boggs and Morris [9] in 1909. Since then lipemia in rabbits has been similarly produced by Milne,[10] Sakai,[11] Horiuchi,[12] Fishberg and Fish-

9. Boggs, T. R., and Morris, R. S.: J. Exper. Med. **11**:553, 1909.
10. Milne, L. S.: Deutsches Arch. f. klin. Med. **109**:401, 1912-1913.
11. Sakai, S.: Biochem. Ztschr. **62**:387, 1914.
12. Horiuchi, Y.: J. Biol. Chem. **44**:345, 1920.

berg,[13] Fishberg,[14] Heki [15] and Johansen.[16] None of these workers were ever able to produce edema in their rabbits. Fishberg and Fishberg suggested that there might be some retention of water in their animals even though edema was never demonstrable. As the bleeding was continued, the lipemia became more marked, but at no time did it reach or maintain a constant level. On the whole, the plasma lipoid level was very irregular throughout. A milky plasma did not always indicate a high lipoid content and vice versa. In all cases, the increase was primarily in the neutral fat (saponifiable) fraction. Sakai, Horiuchi and Fishberg and Fishberg made the same observation. The sterol (nonsaponifiable) fraction showed only a moderate increase as a rule. Occasionally, however, there was a sudden marked rise.

The total plasma protein was slightly decreased as the bleeding progressed. The lowest value encountered was 4.51 per cent (rabbit 1), but this was only transitory and rapidly increased to 7.67 per cent. In the other three animals, the total plasma protein never fell below 5 per cent. Occasionally it rose to 8 and 9 per cent.

In all cases, the albumin showed a constant and fairly uniform decrease. There was a simultaneous increase in the globulin. The albumin-globulin ratio was reversed for the most part. In rabbits 1 and 4, in which edema developed, the final values for albumin fell to 1.87 and 1.88 per cent, respectively. The edema, however, came on when the albumin was approximately 2.5 per cent, and in rabbit 4 even rose to over 3 per cent just when the edema was becoming marked. Similar figures for albumin (2.5 per cent) in rabbits 2 and 3 were constantly found, yet these animals never showed edema. The lowest percentage of albumin (1.61 per cent) was found in a nonedematous rabbit (no. 3).

The total nonprotein nitrogen showed a terminal increase in the edematous rabbits (1 and 4). It remained within normal limits in the nonedematous animals (2 and 3).

A marked secondary anemia developed in all the animals, although this came on quicker and became more pronounced in some animals than in others.

All four rabbits lost weight and became greatly emaciated as the bleeding progressed. This was especially noticeable in the nonedematous ones. They became listless, and usually began to have a watery diarrhea a few days before death.

The urine at times showed a small amount of albumin after the onset of the lipemia. This was seldom more than a trace and was

13. Fishberg, E. H., and Fishberg, A.M.: Proc. Soc. Exper. Biol. & Med. **25**:296, 1927.

14. Fishberg, E. H.: J. Biol. Chem. **81**:205, 1929.

15. Heki, M.: J. Biochem. **11**:369, 1930.

16. Johansen, A. H.: J. Biol. Chem. **88**:669, 1930.

always inconstant. Rabbits 1 and 3 on one occasion showed a few white blood cells, red blood cells and granular casts. Fat appeared in the urine as the lipemia progressed. It showed about the same values for saponifiable and nonsaponifiable fat as did the fat in the plasma.

At autopsy all animals were greatly emaciated. The subcutaneous mesenteric and perirenal fat was almost gone. Boggs and Morris, Sakai, Horiuchi, Fishberg and Fishberg and Johansen found the same condition in their rabbits. The viscera were pale and anemic. In rabbits 1, 2 and 4 there was a well marked fatty metamorphosis of the liver, the fat being most prominent at the periphery of the lobules. The surfaces of the kidneys were smooth and golden yellow. On section, the medullae were very pale, while the cortices, especially the peripheral parts, showed the same golden yellow color as the surface. The bone marrow, with a single exception (rabbit 3), was red only at the ends of the long bones.

In addition to the aforementioned findings, rabbits 1 and 4 showed

TABLE 5.—*Analyses of Edema Fluids*

Rabbit	Total Protein, per Cent	Albumin, per Cent	Globulin, per Cent	Nonprotein Nitrogen, Mg. per 100 Cc.	Source of Fluid
1	5.77	1.73	4.04	125	Ascitic fluid
	2.44	1.10	1.34	81	Subcutaneous fluid
4	3.51	1.57	1.94	146	Ascitic fluid
	3.65	1.06	2.59	72	Subcutaneous fluid

a massive edema of the scrotum, massive ascites and hydrothorax. There was also a moderate subcutaneous edema of the hind legs in rabbit 1. The subcutaneous tissue measured from 0.5 to 1.5 cm. in thickness, and displayed a glassy gelatinous appearance. A clear, watery fluid could be easily expressed by simply squeezing the tissue.

Analysis of the subcutaneous fluid showed it to be fairly high in protein (table 5). The protein content of the ascitic fluid was likewise high (table 5). In all the edema fluids, the globulin was in excess of the albumin, and in general reflected the protein changes in the blood plasma.

Rabbit 3 (nonedematous) was bled over a period of nearly two and one-half months with a total blood loss of 2,105 cc. Unlike the other three animals, this one showed no gross evidences of fatty changes in the liver. Subsequent analysis of the liver showed the fat content to be entirely within normal limits (table 6). The bone marrow was red throughout the shafts of the long bones.

The kidneys of all four rabbits were analyzed for water and lipoid content (table 7). In addition, the livers of rabbits 3 (nonedematous) and 4 (edematous) were similarily analyzed (table 7). For comparison, two normal rabbits were included.

The kidneys of the edematous (1 and 4) rabbits showed a slight increase in water and a rather marked increase in lipoids. Conversely, the kidneys of the nonedematous animals (2 and 3) showed a normal water content, and the total lipoids, although slightly higher than the normal, were lower than those found in the edematous animals. The striking difference was in the saponifiable and nonsaponifiable fractions. In the edematous group, the nonsaponifiable fraction fell from the normal of from 63 to 70 per cent to 1 and 5 per cent. There was a great increase in neutral fat. One of the nonedematous rabbits (2)

TABLE 6.—*Analyses of Fats in Liver*

Rabbit	Fresh Liver, Gm.	Dried Liver, Gm.	Water, per Cent	Fat,* per Cent	Saponifiable Fat, per Cent	Nonsaponifiable Fat, per Cent
Normal	9.09	2.84	69	14	94	6
4†	10.57	2.56	76	36	97	3
3	12.85	3.14	75	19	92	8

* Calculated on the dry weight. † Edematous.

TABLE 7.—*Analyses of Fats in Kidney*

Rabbit	Fresh Liver, Gm.	Dried Liver, Gm.	Water, per Cent	Fat,* per Cent	Saponifiable Fat, per Cent	Nonsaponifiable Fat, per Cent
Normal	17.62	3.89	78	13.6	37	63
Normal	4.98	1.81	77	24.0	30	70
1†	12.81	2.23	83	38.0	99	1
4†	11.25	2.17	81	37.0	95	5
2	5.93	1.40	76	25.0	40	60
3	4.38	0.93	79	30.0	81	19

* Calculated on the dry weight. † Edematous.

showed practically normal values for all the lipoid fractions. In rabbit 3 (nonedematous), however, the nonsaponifiable fractions fell to 19 per cent.

Identical analyses were made on the liver of one edematous (4) and one nonedematous (3) rabbit. Both showed a slight increase in water, but only the edematous animal had an increase in total lipoid. There was nothing significant in the saponifiable and nonsaponifiable fractions.

Hematoxylin-eosin sections of the kidneys of rabbits 1, 2 and 3 were essentially normal. The kidneys of rabbit 4 showed some tubular destruction (especially of the convoluted tubules) and a moderate round cell infiltration into the interstitial tissue. In certain places there was a slight increase in the interstitial fibrous tissue. The glomeruli appeared normal. This was clearly a case of spontaneous nephritis in the rabbit.

Fresh frozen sections of the kidneys of all animals stained with sudan III showed the cytoplasm of the convoluted tubules somewhat overstained with sudan III, but no distinct fat droplets were ever

demonstrable. These sections were compared with sections of normal kidneys subjected to the same stain in all cases.

Sections of the liver stained with hematoxylin-eosin and fresh frozen sections stained with sudan III all showed a marked increase in fat.

COMMENT

There was a marked difference in the reaction of the different rabbits. In general, however, they fell into two groups: the one in which a moderate amount of bleeding was soon followed by a marked edema; the other in which even prolonged bleeding failed to produce edema. The anemia was equally marked in each case. The animals in which edema developed showed a sharp rise in plasma lipoid just when the edema became demonstrable, and the lipoid continued to be elevated while the edema was present. Discontinuance of the bleeding caused both the lipemia and the edema to disappear (rabbit 1). Rabbit 2 (nonedematous) showed low plasma lipoid values for the most part, but at times there would be a sudden rise just as marked as in the edematous animals. In the other nonedematous rabbit (3) lipemia was very slow in developing, but once established it became the most marked of all. Changes in the total concentrations of total plasma protein, albumin and globulin were practically the same in both the edematous and the nonedematous rabbits. The actual amount of total plasma protein removed ranged from 39 to 148 Gm. Both the total amount of plasma protein removed and the resulting plasma protein concentration must, of course, be interpreted in the light of the decreased blood volume. Edema became demonstrable while the concentration of total plasma protein was still within normal limits, and while the concentration of albumin was around 2.5 per cent. It is true that the edematous animals showed terminal albumin concentrations of 1.87 per cent and 1.88 per cent, but the edema had been present for days before this. An inverse relationship between the concentrations of total plasma protein and albumin and those of plasma lipoids as described by Fishberg was generally present. Blood volume was decreased to about the same degree in both the edematous and the nonedematous animals. It would appear, therefore, that the development of edema in rabbits is not directly related to the concentration of total plasma protein or to that of albumin or to that of lipoids. The osmotic pressure of the plasma under such conditions might offer a clue.

The possibility of the repeated heart punctures causing the edema is ruled out by the fact that edema never developed in two of the animals, even though in one of these (rabbit 3) the heart was punctured twice as often as in any of the rest.

The edema fluids of the rabbits were exceptionally high in protein (table 5). In this they were in direct contrast with the edema fluids of dogs in which the edema was produced by plasmapheresis. When bleeding alone was used to induce the edema, there was an actual depletion of water in the organism. Oddly, the animals did not manifest an increased thirst under such conditions. The rabbits used derived most of their water from the fresh vegetables that were fed them and drank little. In the edema of dogs produced by plasmapheresis, water was continuously added in the form of Ringer's or Locke's solution. Large amounts of water were also given by stomach tube by some of the workers. This added amount of water may explain a part of the difference in the protein concentration in the edema fluids.

It has already been suggested that it is difficult to explain the development of edema in a rabbit that is suffering a continuous loss of water on the observed alterations in the concentrations of the plasma proteins and lipoids. It must be assumed that there is some increased affinity or "thirst" of the tissue colloids for water. Without this assumption it is difficult to understand how the tissues could draw water from the blood and hold it when the blood volume is depleted and no extra fluid is added in any way.

From the comparative analyses of the liver and kidneys of normal rabbits and those with a marked and prolonged lipemia it would seem that the excess fat in the kidneys and in the liver is probably derived from the blood. The increase here, as in the blood plasma, is primarily in the neutral fat fraction.

The reduction in the nonsaponifiable (sterol) fraction with the simultaneous increase in the neutral fat portion in the kidneys of the edematous rabbits is striking (table 6). A satisfactory explanation of this shift is difficult to offer. It is practically impossible to assume from the known chemical structures of the fatty acids, neutral fats and sterols that there could be a conversion from a sterol to a neutral fat. From the given experimental data it is impossible to say whether this bears any relation to the development of the edema.

It was interesting to note that while fresh frozen sections of the kidney stained for fat failed to show stainable fat, the chemical analysis demonstrated a marked increase. The fat in the liver was always stainable.

It is probable that the excess fat in the plasma was derived from the fat depots of the body. The fact that the increase in plasma lipoid was primarily in the neutral fat fraction gives support to this theory. In all cases of marked lipemia, the rabbits at autopsy showed a striking decrease in subcutaneous, mesenteric and perirenal fat. At times there was practically no demonstrable fat. Similar observations were reported by Boggs and Morris, Sakai, Horiuchi, Fishberg and Fishberg and Johansen.

A satisfactory explanation for the accumulation of fat in the blood has never been offered. Boggs and Morris suggested that the lipemia was due to defective oxidation secondary to the anemia. Griffel [17] was able to induce a lipemia in rabbits by letting them live for a time in rarefied air. Sakai found a decrease in blood lipase and suggested this as the cause for the excess fat in the blood. Fishberg thought it was secondary to the loss of plasma protein.

It is interesting to note in this connection that it is very difficult, if not impossible, to induce lipemia in rabbits when phenylhydrazine (Heki) or pyrodine (Boggs and Morris) is used to bring on the anemia.

Some of the excess plasma fat is excreted as such by the kidney and can be recovered in the urine. This excretion of fat by the kidney goes on for the most part without any demonstrable histologic changes in the kidney. It is true that the kidneys of edematous rabbit 4 showed some tubular destruction with some round cell infiltration, but this obviously was a spontaneous nephritis in the rabbit, a disease to which rabbits are prone. There is no direct evidence that it was secondary to any experimental procedure.

A long-standing edema cannot be produced in the rabbit by simple bleeding, because the animal always dies as the bleeding is continued. On the other hand, if the bleeding is discontinued after the edema has become marked, the edema at once subsides and the rabbit recovers.

Relationship of Experimental Edema and Lipemia to Lipoid Nephrosis.—There are some points of resemblance between this experimental disease of rabbits and human lipoid nephrosis. Both show edema, lipemia and a decrease in plasma albumin.

In the lipemia of rabbits, the increase in plasma fat is primarily in the neutral fat fraction. In lipoid nephrosis, the increase in plasma lipoid is likewise in the neutral fat portion (unpublished work).

In lipoid nephrosis, the patient loses plasma protein, especially albumin, through damaged glomerular capillaries. In the experimental work here reported, plasma protein was removed by bleeding. In both cases, the albumin is greatly reduced, while the globulin is markedly increased in the rabbit and normal or slightly increased in man. The rabbits in this series did not show as great a reduction in the percentage of total protein as is generally encountered in lipoid nephrosis. However, if one takes into consideration, the decrease in blood volume, there is a marked decrease in total plasma protein even though the concentration at the time failed to show a striking decrease. The albumin and globulin concentrations must be similarly interpreted.

17. Griffel, W.: Biochem. Ztschr. **222**:290, 1930.

Whether or not there is any etiologic relationship between the experimentally produced edema in rabbits and the edema of lipoid nephrosis must remain an open question for the time being. The experimental work suggests that the lipemia and perhaps the edema are caused in some way by the loss of plasma protein in both cases. The resulting concentrations of plasma protein and plasma albumin in the edematous rabbit are much higher than in the patient with lipoid nephrosis, even though there is a decrease in the actual amounts of the total protein and albumin. It is difficult, therefore, to explain the sudden onset of the edema in rabbits on the basis of an altered concentration of plasma protein or of albumin. An increased affinity for water on the part of the tissue colloids would help to explain the transfer of water and the resulting edema. How a loss of plasma proteins brings about lipemia and edema remains to be determined.

The urine of the rabbits was always high in fat. The protein content of the edema fluids of the rabbits (table 5), especially the subcutaneous fluid, was considerably higher than that usually described as typical of lipoid nephrosis. It was likewise higher than the protein content of the edema fluids in the dogs described by Leiter and by Shelburne and Egloff, in which edema was produced by plasmapheresis.

The histologic structure of the kidneys in these experimental animals was entirely different from that of the kidneys in human lipoid nephrosis.

CONCLUSIONS

Edema was produced in two of four rabbits by prolonged bleeding alone.

All rabbits showed a marked and persistent lipemia. The increase in plasma lipoid was primarily in the neutral fat fraction, the sterol fraction as a rule being less increased.

Total blood volume and plasma volume fell as the bleeding progressed. This occurred in both the edematous and the nonedematous animals, and was about the same in each group. The concentration of total plasma protein fell but slightly; that of the albumin was moderately lowered and that of the globulin at times greatly increased. Reversals of the albumin-globulin ratio were frequently encountered.

The edematous rabbits showed a marked edema of the scrotum and anterior abdominal wall, massive ascites and hydrothorax. All edema fluids were high in protein.

The urines of all the animals, both edematous and nonedematous, were high in fat. This was mostly neutral fat. There was a variable increase in the lipoid content of the kidney and liver in all instances. In the kidneys of the edematous rabbits there was a striking decrease in sterols, with an equally marked increase in neutral fat.

Laboratory Methods and Technical Notes

A RAPID METHOD FOR STAINING FROZEN SECTIONS*

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This method of staining combines the use of a rapid and permanent nuclear stain and that of a counterstain. It has been found extremely valuable in the operating room and in routine diagnostic study of frozen tissues.

TECHNIC

Apparatus and Reagents.—Apparatus and materials to be used are: Spencer freezing microtome and accessories, microscope, five dropping bottles (capacity 30 cc.), two petri dishes, one porcelain dish, tripod, bunsen burner, one glass rod teaser, fine blotter, coverslips, glass slides, 80 per cent alcohol, 95 per cent alcohol, clearing and dehydrating solution, iron hematoxylin, alcoholic solution of eosin, Canada balsam and 10 per cent formaldehyde.

Preparation of Stains and Clearing Solution.—The iron hematoxylin solution is a modification of Weigert's iron hematoxylin. It is prepared as follows: Stock solutions A and B are first prepared.

Stock Solution A: Hematoxylin (Grübler), 1 Gm.; 95 per cent alcohol, 100 cc.

Stock Solution B: Ten per cent ferric chloride (Merck), 5 cc.; glacial acetic acid, 15 cc.; distilled water, 80 cc.

Both stock solutions are to be kept in a dark place. It is preferable that solution A be allowed to age for from two to three weeks before using. It should be discarded after six months.

The nuclear stain is prepared by mixing well 2 parts of solution A with 1 part of solution B. It is allowed to stand about one hour before using. The indicated proportions must be carefully measured, as a slight variation in the concentrations of the acid and ferric chloride tends to render the stain less stable. When solutions A and B are mixed, an intense blue color develops that gradually assumes a deep purple, and finally a deep red-brown, color. If carefully prepared, this solution is ready to use within an hour and retains its staining properties well for from three to four weeks. If a green color develops in the stain, it is unfit for use. This color is due either to the use of unclean glassware or to too great concentrations of acetic acid and ferric chloride. It is preferable that the stain be kept in a dark amber bottle with a ground glass stopper.

The counterstain is prepared as follows: Dissolve 2.5 Gm. of water-soluble yellow eosin in about 10 cc. of distilled water and make up to 500 cc. with distilled water. Add 4 cc. of concentrated hydrochloric acid, drop by drop. A precipitate forms, which settles to the bottom. Pour off the supernatant fluid and wash six

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times with distilled water. Then filter and allow to dry in the oven at about 100 F. for twenty-four hours. Scrape off the residue from the filter paper and place it in the bottle. To each 0.5 Gm. add 100 cc. of 80 per cent alcohol. This solution keeps permanently.

The dehydrating-clearing solution is prepared after the method of Kumagai[1] and consists of the following reagents:

	Cc.
Xylene	7.5
Phenol	2.5
Absolute alcohol	10.0

Staining.—Very thin sections of tissue are fixed in 10 per cent formaldehyde for twelve hours, if time permits, or for three minutes in boiling 10 per cent formaldehyde, if a rapid diagnosis is required. The technic of sectioning frozen tissue described by Broders[2] has been found very satisfactory. It is recommended that the reader refer to this article, since many points are brought out that are of importance in the technic of obtaining frozen sections. The rapidity of this method rests almost entirely on the efficiency and speed of obtaining sections, as the staining procedure requires less than one minute. With proper technic, sections may be obtained that are not greater than 10 microns in thickness. Thick sections are not satisfactory, as proper dehydration and clearing are not easily obtained.

The sections are placed in shallow petri dishes containing water. A thin section is selected and floated on the surface of a clean glass slide. The section is then spread out by dropping 80 per cent alcohol directly over its surface. In this manner, practically all of the wrinkles in the section may be removed. The excess alcohol is drained off, and a blotter is placed firmly over the section, with the use of a moderate amount of pressure to avoid displacement. In this manner, it has been found that the section adheres closely to the slide and is not removed by blotting, whereas if the section is blotted without first using alcohol, it very often adheres to the blotter. Care must be taken to allow as little time as possible to elapse between blotting and staining, so that drying of the tissue is prevented.

The following procedure is employed in staining the section:

1. Cover the section with iron hematoxylin and allow it to stain for from five to ten seconds.
2. Wash off with 80 per cent alcohol.
3. Cover the section with eosin.
4. Wash immediately with 80 per cent alcohol.
5. Wash quickly with 95 per cent alcohol.
6. Treat with a few drops of dehydrating and clearing solution.
7. Mount in balsam.

The stain and other fluids are dropped directly on the section. In dehydrating and clearing, the slide is held at an angle, and the reagents are allowed to drain into a petri dish. From 10 to 15 drops of the alcohols and dehydrating and clearing reagents are sufficient. From 4 to 5 drops of the stains are ample for the section of average size.

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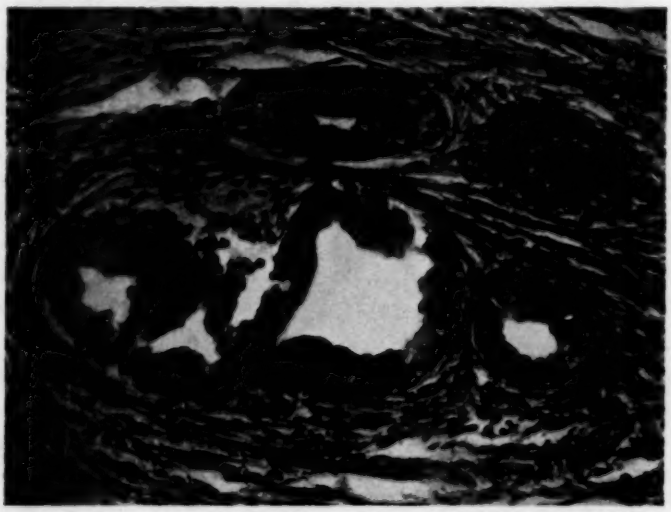
1. Kumagai, Kuranosuke: Folia anat. japon. **3**:31, 1925.
2. Broders, A. C.: J. Lab. & Clin. Med. **16**:734, 1931.

COMMENT

By using proper concentrations of iron hematoxylin and eosin, and a short exposure of the tissues to the stain, it has been found that differentiation, other than that obtained by the use of the alcohols and the dehydrating and clearing solutions, is not necessary. The time required for this stain is therefore much shortened. Clear nuclear staining may be obtained in tissues that are cut thin and properly fixed. The accompanying photomicrograph shows the fine detail of cytoplasm and nucleus obtainable by this method. The nucleus is of a deep blue color with stains that are from one hour to one week old. With



Hyperplasia of prostate; × 80; frozen section, stained with iron hematoxylin and eosin.

older stains, the nucleus is of a blacker color. The cytoplasm is clearly defined and purplish red.

If sections are thin, proper clearing is easily accomplished by this method, and the sections retain their color permanently. If sections are thick, clearing may be continued after examination of the slide by removing the balsam and allowing the sections to remain in the xylene for several minutes.

The use of Kumagai's clearing and dehydrating reagent has been found very satisfactory, as proper clearing is accomplished without previous dehydration with absolute alcohol. Also much of the shrinkage that is produced by the use of xylene alone is avoided.

General Reviews

SYPHILITIC MYOCARDITIS *

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(Concluded from page 295)

HISTOLOGIC CRITERIA OF SYPHILITIC MYOCARDITIS

In reviewing the literature casually one gains the impression that syphilitic myocarditis is by no means rare. Seemingly many cases are reported, and one may therefore be tempted to agree with Warthin, who stated that old latent syphilis is one of the most important causes, if not the most important cause, leading to myocardial incompetency. A closer and more critical inspection of the literature on this subject, however, reveals that in many cases either the diagnosis "syphilis" or the diagnosis "myocarditis" was obviously not based on histologic facts. Changes in the myocardium found incidentally in cases of syphilis in other organs are often interpreted as syphilitic in origin because of the evidence of syphilis elsewhere. On the other hand, fibroses of the myocardium as the result of vascular disturbances are often thought to be remnants of myocarditis.

In the following paragraphs I shall try to give the histologic criteria of syphilitic myocarditis in cases reported in the literature.

Virchow stated that one finds in the heart in cases of syphilis multiple fibrous scars without any other demonstrable cause but syphilis. He mentioned, however, that it is difficult to prove that such lesions are syphilitic. Mueller believed that four of his reported cases showed syphilitic myocarditis, because there was no other etiology for the myocarditis found at autopsy, because the same type of fibrosis is also found in hearts that show gummas, and because of the similarity of the changes in the myocardium in his cases and the changes found in syphilitic orchitis and interstitial syphilitic hepatitis. Ehrlich, probably the first to describe endarteritic changes of the coronary vessels, believed that the fibrous myocarditis in the case that he reported was the result of those vascular changes. Histologically, the myocardium showed necrosis, a marked cellular infiltration, many capillaries and a more or less prominent invasion of pus cells. The myocarditis in

Chvostec's case was thought to be of syphilitic origin because of the coincidental syphilitic lesions in the brain. Mracek believed that the combination of old and newly formed connective tissue with granulation tissue is characteristic of syphilitic myocarditis. He also stated that the diagnosis of syphilis can be made only by the analogy of the changes to syphilitic changes in other organs. Loomis said that syphilis gives rise to an indurated myocarditis, a condition that in its later stages is hardly distinguishable from fibrous disease due to other causes. It has been possible to infer the origin of this fibrosis only from the antecedent history of the patient. Stolper found in his cases a cellular infiltration of the adventitia of the smaller branches of the coronary arteries and endarteritis obliterans. Herrick stated that the fibrous form of myocarditis is characterized by grayish areas or streaks in the myocardium. These areas when fully fibrous differ in no respect from fibrous myocardial patches due to coronary obstruction with consequent myomalacia and subsequent scarring. He mentioned that the diagnosis of syphilitic myocarditis must be based on probability. Adler's case showed a gumma in the suprarenal gland; the heart was the seat of a cellular infiltration of the myocardium with some coagulation necrosis, but no vascular changes. Wagner and Qwiatkowski based their diagnosis of syphilitic myocarditis on the finding of much fibrous tissue in the midst of the muscle tissue, with many spindle-shaped cells, necrotic portions and endarteritis of branches of the coronary arteries. Herxheimer stressed the point that an infiltration of round cells of the interstitial tissue along the blood vessels is characteristic of syphilitic fibrous myocarditis; scar tissue, mainly in the left ventricle close to the apex, is also often encountered. Landois found much connective tissue in the myocardium with only a few nuclei. There were cells surrounding small blood vessels, the lumina of which were obliterated; the larger vessels showed no changes. Takata's case revealed circumscribed perivascular infiltrations, mainly of round cells and plasma cells; there was a new formation of connective tissue present with some polymorphonuclear leukocytes. Some of the smaller arterioles and venules showed a thickened intima. He believed that circumscribed infiltrations mainly of plasma cells about the venules are characteristic of syphilitic myocarditis. Spalding and von Glahn based their diagnosis on the finding of necrosis and polymorphonuclear leukocytes and on the presence of a moderate number of spirochetes. Hines found recent and old infarcts in the heart; histologically, granulation tissue with polymorphonuclear leukocytes, a perivascular infiltration by lymphocytes and some fibrous scarring were present. He also mentioned finding spirochetes. Boyd found lymphocytes and plasma cells in the adventitia of the coronary artery, and in addition polymorpho-

nuclear leukocytes. He also claimed to have found spirochetes. Wilson reported patches of sclerosis and an infiltration by round cells in the interstitial tissue of the myocardium; spirochetes were found in the interventricular septum. Gravier found much interstitial connective tissue with plasmatocytes and edema. Many capillaries were found in some portions and much fibrosis in other fields. Chaniotis found a diffuse fibrous myocarditis consisting histologically of connective tissue, degenerative changes of the muscle fibers and edema.

The criteria of syphilitic involvement of the myocardium as given by Warthin will now be referred to in detail.

In 1914 he described the cardiac lesions produced by Spirochaeta pallida. He stated that it had long been suspected that syphilis played an important rôle in the production of cardiac disease. The truth of the matter was, he said, that before the discovery of Spirochaeta pallida as the etiologic agent of syphilis, pathologists recognized but few lesions of the heart as essentially pathognomonic of syphilis. As to the significance of the gumma, there were no doubts, but gumma of the heart was relatively rare, and actual proof of cardiac syphilis was restricted to the instances in which the gumma was present. The frequent association of forms of chronic myocarditis, fibrous heart, anemic infarction of the myocardium and coronary sclerosis, with other pathologic evidences of syphilis elsewhere in the body (such as tabes, dementia paralytica, gumma of the brain or of the liver, aortic aneurysm, lesions of the bones, orchitis fibrosa, etc.) had given pathologists a strong leaning toward the view that the heart was one of the organs most frequently affected in syphilis. That these lesions were actually syphilitic could be assumed only on the strength of circumstantial evidence before the demonstration of Spirochaeta pallida. Warthin claimed to have found large colonies of spirochetes in the myocardium, either in the tissue spaces of certain muscle areas or about the blood vessels, without any changes in the adjacent cardiac muscle that could be recognized by any of the technical methods employed at the present time. Studies of cardiac syphilis showed, he said, that the primary lesions produced by the spirochetes may be either parenchymatous or interstitial. The parenchymatous lesions are a peculiar pale degeneration, fatty degeneration, simple atrophy and necrosis; the interstitial lesions are a peculiar form of edema (myxedema), vascular and perivascular infiltrations and localized myxoma-like formations. The parenchymatous changes may occur absolutely independently of the interstitial, and the latter may be found with no associated changes in the adjacent cardiac muscle. The more marked the interstitial changes, the more likely are parenchymatous changes to be associated with them, but the most marked parenchymatous lesions may occur without any interstitial changes. The purely parenchymatous lesions

are found especially in virulent congenital, active secondary and early tertiary syphilis. In milder and older infections the interstitial changes, particularly the localized vascular and perivascular proliferations, predominate. The myxoma-like formations resembling undifferentiated gummas also occur in more localized and milder infections. It is also of great importance to know that the heart is so frequently the seat of localization of spirochetes. The cardiac localization of spirochetes is more common than the hepatic. Spirochetes may be found in great numbers in the heart when they can be found nowhere else in the body. In such cases the cardiac muscle may also show no lesions that, according to older knowledge, would be classed as syphilitic; indeed, no organ or tissue may show any histologic sign of syphilis even when the organisms are present in great numbers.

In 1918, in his "New Pathology of Syphilis," Warthin described the myocardium in syphilis. To the naked eye, the hearts show, as a rule, dilatation, hypertrophy, atrophy and fibroid patches in the wall of the left ventricle. The determination of cardiac syphilis is essentially microscopic. In syphilis, a progressive fibrosis takes place, which in some cases extends through the entire myocardium. The fibroid heart is the ultimate outcome of all cases of latent syphilis. The striking feature of the fibroid area is the dilatation of preexisting capillaries or veins or a new formation of such in the fibroid area. Such areas often appear cavernous or sinusoidal. The vascular proliferation appears to be one of the distinct features of syphilitic myocarditis. In more acute cases, the myocardium is edematous, often giving a slight reaction for mucin. In a great majority of cases, the myocardium shows healed fibroid areas in association with the active infiltrations. Warthin further stated that the essential lesion is the interstitial myocarditis characterized by lymphocytes and plasma cells infiltrating along the blood vessels between the muscle fibers. The infiltrations usually are patchy or diffuse, very rarely focal or circumscribed, thus differing from those caused by an infection with Streptococcus myocarditis. Polymorphonuclear leukocytes are few in these infiltrations. No eosinophils are present, but mainly histiogenic lymphocytes and young formative cells. Giant cells are rare. The large epithelioid fibroblast is common. The entire cardiac wall from the epicardium to the endocardium, including the papillary muscles, may be involved in the infiltrations. In the most severe cases, infiltrations are grouped around the coronary arterioles and many reach such a size as to suggest miliary gummas. A thrombosis of the thickened endocardium overlying the fibroid patches is more frequent than aneurysmal dilatation. In regard to the presence of spirochetes, he stated that in old cases with fibrosis more or less well advanced the demonstration of spirochetes becomes a task requiring patience and determination

spread often over days or weeks. Nevertheless, they are more easily found in the heart than in any other organ or tissue.

In 1925, Warthin reported 8 cases of sudden death due to exacerbation of latent syphilitic myocarditis. In this article, he spoke of active syphilitic myocarditis, of chronic syphilitic myocarditis and of fibroid myocarditis with acute exacerbation. The microscopic features of the cardiac lesions were old fibrosis (completely healed myocarditis), subacute infiltrations by lymphocytes and plasma cells, monocytes and a predominance of polymorphonuclears. Spirochetes were found, particularly in these more acute areas. All stages of syphilitic myocarditis were represented in these lesions. Miliary gummas with giant cells were occasionally found (particularly in a certain case). The coronary changes were relatively slight, except in 1 case in which the anterior left descending branch showed extensive syphilitic arteritis. No Aschoff's nodes were found. Warthin also stated that the important, if not predominant, rôle played by syphilis in the production of myocardial incompetency is not recognized by the major part of the profession, internists as well as pathologists. Old latent syphilis is one of the most important causes, if not the most important cause, leading to myocardial incompetency. The person with latent syphilis in the great majority of cases eventually dies from "cardiac failure." This may be brought about in several ways: (1) myocardial atrophy and fibrosis due to slowly progressive, mild syphilitic lesions in the myocardium (this is the most common form); (2) syphilitic disease of the coronaries with resultant infarction and fibrosis; (3) a combination of these two processes; (4) syphilis of the aortic valve (always associated with some degree of myocardial syphilis); (5) a combination of myocardial syphilis and syphilitic mesaortitis, and (6) acute exacerbations of a previously latent syphilis ("critical stage" of syphilitic infection) or an acute malignant type of cardiac syphilis. This is much less common than the chronic latent form, but its frequency remains to be determined, as it has not received pathologic recognition.

ANALYSIS OF THE HISTOLOGIC CRITERIA OF SYPHILITIC MYOCARDITIS

If one regards the criteria of syphilitic myocarditis critically, so as to rule out any other etiologic agent as the basis for such lesions, the following arguments present themselves.

Myocarditis is classified as parenchymatous and interstitial. Though such a subdivision is not always clearly distinguishable, it can be made in many cases. In cases of interstitial myocarditis, the inflammatory cells are found close to the branches of the coronary vessels, which always lie within the interstitial tissue. As a matter of fact, scar tissue

as the result of healed myocarditis is often confined to the perivascular spaces. It is also known that the place of predilection of Aschoff's nodules is the perivascular space of the interstitial tissue in the myocardium. A perivascular distribution of cells or of fibrous tissue in the myocardium, therefore, is not pathognomonic of syphilitic myocarditis.

It is true that the typical cells in syphilis are the lymphocyte and the plasma cell. But it is also known that in many instances of myocarditis of nonsyphilitic origin, plasma cells and lymphocytes are found. Especially the isolated form of myocarditis (Fiedler's myocarditis) shows, in addition to other cells, endothelial cells, many lymphocytes and plasma cells (Scott and Saphir). As to the finding of round cells in the myocardium, Liebmann found an accumulation of round cells in the perivascular spaces of the myocardium in two cases of pneumonia. As will be shown later, infiltrations by round cells in the myocardium are often found in cases of coronary sclerosis. The diagnosis of syphilitic myocarditis, therefore, should not be based alone on the finding of these varieties of cells.

A diffuse fibrosis of the heart is also found in other conditions. Rheumatic myocarditis results often in diffuse fibrous patches throughout the myocardium. After scarlet fever, typhoid fever, pneumonia and other nonspecific infectious diseases the myocardium may show a diffuse fibrosis. The cases reported by von Glahn and Wilshusen are excellent demonstrations of the coincidental finding of syphilitic aortitis and rheumatic myocarditis in the same case. Huebschmann described marked fibrosis of the myocardium following diphtheritic myocarditis; the fibrosis was so extensive as to be called "cardiac cirrhosis." Similar cases were reported by Schmincke. Besides, it should not be forgotten that chronic toxic processes such as chronic alcoholism, gout, lead poisoning, etc., may lead to chronic interstitial myocarditis (Karsner).

The young granulation tissue often described as occurring in syphilitic myocarditis also is not pathognomonic of syphilis. It is found in cases of coronary sclerosis, and more often in cases of subacute myocarditis occurring in the course of subacute bacterial endocarditis. But it also seems evident that young granulation tissue might easily be found in any case of myocarditis of longer duration.

Sclerosis of the coronary arteries, such as was present in some of the cases recorded in the literature, might have been the cause of some of the fibrosis ascribed to syphilitic myocarditis. Kaufmann mentioned the richness in blood vessels of the granulation tissue in coronary sclerosis. He stated that such fibrosis might be diffuse and therefore spoke of "myocarditis fibrosa disseminata." Karsner also spoke of fibrosis due to progressive arteriosclerosis. The larger branches of the coronary arteries might be practically free from changes while the smaller branches show severe lesions. As was mentioned before, steno-

sis or occlusion of the mouths of the coronary arteries might lead to fibrosis of the myocardium. Such conditions are found especially frequently in cases of syphilitic aortitis. Moenckeberg mentioned that changes in the myocardium sometimes are found in cases in which the coronary arteries offer no changes, and in which only the ascending portion of the aorta is the seat of sclerotic lesions. Moenckeberg stated that a rigidity of the ascending portion of the aorta alone might explain the insufficient blood flow through the coronary arteries and hence myocardial fibrosis. It is known, however, that in some cases a constriction of the mouths of the coronary arteries does not necessarily lead to marked myocardial changes. But there are also cases on record which show myocardial infarction referable only to constriction of the mouths of the coronaries in cases of syphilitic aortitis. Areas of myocardial infarction show, in addition to necrosis, a cellular infiltration, especially of polymorphonuclear leukocytes, as was shown recently by Wearn. Such a case will be mentioned later.

It is true that endarteritis obliterans is very characteristic of syphilis, but by no means pathognomonic. It also is observed in chronic inflammations of nonspecific origin. Besides, in the cases recorded in the literature in which the diagnosis of endarteritis was made, it does not follow from the descriptions that this was really primary endarteritis obliterans and not merely the end-stage of arteriosclerotic intimal thickening in small vessels resulting in obliteration of the lumina.

Fatty degeneration, atrophy, necrosis and edema are not characteristic of syphilis. In view of the finding of necrosis, it might be mentioned that Bracht and Waechter have shown such an occurrence in their experimental studies with intravenous injection of diplococci and streptococci. Kaufmann mentioned finding necrosis and fatty degeneration of the myocardium in cases of acute interstitial myocarditis. Edema and mucoid degeneration were described in a previous paper (Saphir and Scott) as resulting from vascular changes, and as such they are not characteristic of syphilis.

In the cases of syphilis with gummas in the myocardium, or at least accumulations of cells suggesting miliary gummas, with giant cells, the syphilitic nature of the myocarditis seems established.

Summarizing the histologic criteria of chronic syphilitic myocarditis, I must state that none of the criteria is sufficient to allow the making of such a diagnosis. I do not believe that without knowing the history of the case and without being told that the Wassermann reaction was positive, or that the patient had other signs known to be those of syphilis, the diagnosis of syphilis could have been made from the histologic lesions of the myocardium alone in any of the cases reviewed.

SUMMARY OF EVIDENCE AGAINST THE DIAGNOSIS OF SYPHILITIC MYOCARDITIS

Hertz as early as 1873 doubted the diagnosis of syphilitic myocarditis in the case that he reported, even though an aneurysm of the aorta and so-called syphilitic pneumonia were present. Ziegler in 1887 stated that many cases reported in the literature as cases of syphilitic fibrous myocarditis are more likely cases of myocardial fibrosis on an arteriosclerotic basis. Curschmann mentioned that to the time of his report in 1893 there were no definite criteria allowing a differentiation between syphilitic proliferation of connective tissue and fibrosis from other causes. Stolper preferred to speak of fibrous myocarditis in syphilitic persons rather than of syphilitic myocarditis. Quensel remarked that fibrous myocarditis found in the heart in cases of syphilis offers nothing characteristic of syphilis either grossly or histologically. Berblinger was of the opinion that histologically one cannot differentiate between simple fibrous myocarditis and fibrous myocarditis as the result of gummatous myocarditis. In the discussion of Benda's paper it was brought out that every author had confessed that he was unable to find specific signs of syphilitic myocarditis. Bloch stated that no pathologist is able to make the diagnosis of syphilitic fibrous myocarditis in a given section. Moenckeberg and also Scott were not convinced that a fibrous syphilitic myocarditis exists. Stokes stated that postmortem studies have given to syphilis of the aorta a definiteness that unfortunately does not apply to gross syphilis of the myocardium. Cowan and Faulds recently stated that the ultimate lesions in the cardiac muscle are not syphilitic, though they have a syphilitic cause; that is, they are the result of occlusion of the mouths of the coronary arteries.

Ehrlich, Orth, Mracek, Kockel and Krehl believed that in syphilis primary vascular changes may lead to the histologic picture of fibrous myocarditis. Sears also stated that syphilis of the heart is essentially syphilis of the blood vessels. Brooks stated that in 35 of his cases of syphilis the changes of the coronary arteries were relatively greater than the general arterial changes. On the other hand, in my opinion, Brooks offered no proof that these changes were syphilitic.

Without discussing clinical reports of syphilitic myocarditis, I must mention that in many instances anatomic reports refer to such cases as if they were proved cases of syphilitic myocarditis. In the great majority the clinical diagnosis is based mainly on a positive Wassermann reaction and an apparent improvement of the patient's condition in the course of antisyphilitic treatment. It also might be mentioned in this connection that many articles labeled "cardiovascular syphilis" refer only to syphilis of the aorta and of the aortic valve and do not

mention involvement of the myocardium. But such a title is apt to create an erroneous conception in regard to the frequency of myocardial syphilis.

One also finds in the literature repeated statements based entirely on clinical evidence, indicating that a syphilitic myocardial lesion may occur without valvular mischief and sometimes without thickening of the superficial arteries. Such statements might be correct from a clinical point of view, but unfortunately are often misunderstood and taken as though proved anatomically.

Brooks maintained that one must be careful in asserting that no specific lesions are present in the myocardium, simply because neither gross nor microscopic changes characteristic of syphilis can be demonstrated by the usual technic. It must be emphasized, however, that the only possible way of recognizing syphilis in the myocardium is by a histologic examination. If such a diagnosis cannot be made by the use of the routine staining methods, one has no right to speak of syphilitic myocarditis, even though other manifestations of syphilis are present. A possible myocardial failure in such cases must be explained in some other way.

It seems unwarranted to base the histologic diagnosis of syphilitic myocarditis in part on clinical findings, as was brought out in the discussion of Maher's paper when Maher, asked if he thought that he could make a diagnosis (of syphilis of the myocardium) from his observations in the absence of a history of syphilis, answered that he did not think he could.

Christian, in discussing Lemann's and Mattes' paper, made the following statement:

> I think that at the present time incorrectly we are regarding them (syphilitic changes) as extremely frequent. . . . One of the most difficult diagnoses before the Wassermann reaction, when the spirochete was not discovered, was a positive diagnosis of syphilis either with the naked eye or microscope. We ought to raise the question whether we are very much better off today after we have had the Wassermann reaction and found spirochetes in association with certain cell reactions in determining what is or what is not syphilis on the sole basis of observing this cell reaction. Though sometimes present in syphilis, it does not follow that syphilis is the sole and only cause. . . . It does not seem to me that the evidence can be accepted as satisfactory of the diagnosis of syphilis. . . . I do not believe a dead patient more frequently has syphilis than a live patient. . . . When we come to cardiac disease, as chronic myocarditis, we find the same incidence of positive Wassermann reaction as in the average for all of our patients; but we do not find spirochetes, except Dr. Warthin. Nobody else does with any degree of frequency in these chronic hearts, and in my judgment, the bulk of the cardiac cases, not associated with aortic insufficiency are not syphilis, and that makes up a very large percentage of the cardiac cases in people over 45 years of age. Nearly everybody who has a valvular lesion of the ordinary

rheumatic or bacterial type, dies before he reaches 45. The other cardiac cases develop later in life and are nearly always myocardial in character and very rarely syphilitic.

It also might be of interest to quote Thorel, who said that it must be admitted that the fibrous myocarditis in cases of syphilis offers nothing characteristic. It is impossible to prove in a given case that the fibroid myocarditis is syphilitic. The author believed that it is a matter of speculation and not of science to designate scar tissue in the heart, even though marked, as syphilitic simply because it is found in cases of syphilis.

The failure to find syphilitic lesions in the heart anatomically in cases in which they were apparently suspected led Renvers to the conclusion that syphilis of the heart may heal without leaving pathologic anatomic residua.

There also are authors who believe that the knowledge of histology and of syphilis is at present insufficient to rule out syphilis as the etiologic agent of the myocardial changes. Curschmann was mentioned before. Loomis stated that as knowledge of syphilitic manifestations becomes more perfect, syphilis will be recognized as an important etiologic factor in the production of lesions in the heart. Grassmann stated that the reason why syphilitic lesions other than gumma are found so rarely in the heart is that there is a lack of recognition of such occurrences. Reid stated that it is disappointing to note that in but 1 of 7 reports of cases in which syphilis appeared to be the cause of the changes in the heart did the pathologist definitely state that the lesions were due to syphilis; in the remainder the description is purely anatomic and contains no comment as to the etiology. Reid apparently did not realize the difficulties that confront the pathologist in regard to syphilitic myocarditis. An anatomic description of such changes without comment as to the etiology is practically all that the pathologist can offer.

There are several reports in the literature indicating that syphilitic changes in the myocardium are not encountered more frequently because sufficient studies are not made and not enough sections are taken from the heart for histologic investigations. Though such an argument does not seem fair, it is often used in the discussion of negative results. Sears remarked that syphilitic lesions in the brain, especially vascular changes, are encountered much more frequently than in other parts of the body because they happen to have been studied more carefully. Lamb stated that syphilitic changes in the heart are not found more frequently because it is unusual to take more than one or two pieces of the cardiac muscle for study. This may be true for the particular laboratory from which Lamb took his material, but such statements

are wrong as generalizations. Warthin, in discussing Clawson's paper, believed that the failure to find evidence of syphilis in the myocardium lies in the insufficient number of sections cut from the myocardium.

FINDINGS OF THE SPIROCHETES IN SYPHILITIC MYOCARDITIS

In practically all of his reports of cases of syphilitic myocarditis, Warthin mentioned the finding of spirochetes. Some of the photomicrographs of spirochetes in these articles present apparently typical spirochetes, while others are doubtful. Lemann and Mattes, Spalding and von Glahn, Hines, Boyd and Wilson also found spirochetes in the heart in cases of acquired syphilis. The photomicrograph of spirochetes accompanying the articles by Boyd and by Spalding and von Glahn are not convincing. Lemann and Mattes, and Wilson, do not show pictures of spirochetes in their articles. Spirochetes that are described as drawn out or as broken up or as remnants cannot be regarded as typical spirochetes. If nothing else is found but remnants of spirochetes, they should be entirely disregarded. Similarly, a description of a cycle of spirochetes such as was recently reported by Warthin, convincing though it may sound, should not, in this day, be taken as a description of spirochetes, and the diagnosis of spirochetes should not be based on such metamorphosed organisms. If the finding of such remnants is taken as a positive finding, the literature quickly will be swamped with reports of the observation of spirochetes in almost every organ in every disease. How easy it is to produce artefacts resembling spirochetes or metamorphosed spirochetes will be shown later.

It must be emphasized in this connection that few pathologists are able to find spirochetes in the myocardium in acquired syphilis, in spite of the fact that it is so easy to find them in the myocardium in congenital syphilis. The methods used in staining the spirochetes in congenital syphilis are irrelevant; spirochetes are found in organs in cases of congenital syphilis by the use of any method. According to Christian, nobody, however, finds spirochetes in the myocardium in cases of acquired syphilis as regularly as Warthin did.

Warthin not only found the spirochetes in the myocardium in syphilis but also stated that they are found more easily in the heart than in any other organ or tissue. The discrepancy between his observations and those of other investigators is striking. It probably does not depend on the number of sections examined and the diligence with which the investigators search for spirochetes, but on the purity or impurity of the stains used,[1] on the exact concentration of hydrogen

1. J. Erdheim, in a personal communication, mentioned that the failure of Warthin's assistant to demonstrate spirochetes in his material in Vienna might be due to the fact that too pure a staining material was used.

ions in the solution used (as was demonstrated by Farrier and Warthin) and on the type of material and its relative age and preservation. Also should be mentioned the difficulties encountered in the differentiation in sections between Spirochaeta pallida, fusiform organisms, Spirillum dentium and, sometimes, artefacts.

It is also of interest to note that the finding of spirochetes in the heart in acquired syphilis is apparently little referred to in the German literature or in the French literature. Bloch in 1923 stated that spirochetes have never been demonstrated in the heart in acquired syphilis. Kirch stated that to 1927 a possible syphilitic origin of fibrous myocarditis in cases of syphilis had not been proved by demonstration of the spirochetes. Lenoble stated that it is possible that the spirochetes might disappear from the myocardium and therefore cannot be demonstrated. For that reason he spoke of "myocardites déshabitées."

Coombs made the following statement:

> We have been told by some observers that this (a few plasma cells and lymphocytes drowned in serum which gives the tissue a dropsical appearance) is an inflammatory reaction excited by spirochetes lying latent in the cardiac wall, and as this is a point on which none but the most experienced observer can offer an opinion, I called on Professor Levaditi last autumn at the Pasteur Institute. He was kind enough to undertake the examination of portions of two hearts in which we had already found aortitis and myocardial fibrosis on a generous scale. He reports as follows: "We have examined the blocks that you sent, by the silver impregnation method, that up till now has given us the most reliable results. We have found it impossible to demonstrate spirochetes, in spite of the presence of aortitis. We shall continue to search and will report if we find anything positive." Coming from so experienced an authority, this verdict has peculiar weight; it is, at all events, clear that if spirochetes are in the myocardium, they are so few as to be very difficult to find.

AUTHOR'S OBSERVATIONS

This study deals with an examination of the myocardium in 130 hearts which, at autopsy, showed syphilitic aortitis and an extension of the syphilitic process to the aortic valves. The material was taken in part from the Cleveland City Hospital and in part from the Department of Pathology of Michael Reese Hospital; a few hearts were received from the Cook County Hospital. Blocks of myocardium were taken from various portions of each heart, but especially from the apical portions of the left ventricle and the adjacent septum. At least 15 blocks were taken; but in many cases as many as 60 or 70 blocks were examined. The sections were stained routinely with hematoxylin-eosin. The Levaditi and Warthin-Starry stains were applied for spirochetes. The age of the patients varied from 23 to 65 years. The age of the majority of the patients was about 40 years.

Gross Appearance of the Myocardium.—The myocardium in many cases showed no gross changes. In some cases small grayish dots

and streaks of fibrosis were demonstrable in the myocardium, while only a few cases revealed larger, pearly white plaques of fibrosis. One of the cases, that of a 29 year old woman who died suddenly, revealed a recent infarction of the myocardium, involving the apical portion of the left ventricle and extending into the adjacent septal portions. The endocardium in this region was covered with a recent thrombus. There was an early fibrinous pericarditis corresponding to the infarcted area. The mouth of the left coronary artery was completely occluded, and the mouth of the right coronary was markedly encroached on, but no thrombus was found in any of the coronary branches.

None of the cases showed gummas in the myocardium.

In 41 cases the mouths of the coronary arteries showed constriction. In 11 cases, the constriction was confined to the orifice of the right, and in 10 to that of the left, coronary artery. In 20 cases, the mouths of both coronary arteries were encroached on. In 4 cases there was complete obliteration of the mouth of the left coronary artery, but only in 1 case was there complete obliteration of the mouth of the right. The coronary vessels themselves showed a varying amount of arteriosclerosis—simple intimal thickening, hyalinization and calcification; but there was no gross evidence of syphilis seen throughout their course.

All of the hearts were hypertrophic. The hypertrophy is easily understood on taking into consideration that only those hearts were used that showed in addition to the aortitis, a syphilitic involvement of the aortic valve with insufficiency of this valve.

Histologic Changes in the Myocardium.—The cardiac muscle fibers were larger than normal. The nuclei showed square ends. The striations of some of the fibers were obscured. In some fields a moderate amount of fatty degeneration was found, while others showed some edema between the cardiac muscle fibers, but more pronounced in the perivascular spaces. Many hearts showed a varying amount of connective tissue, in part interrupting the course of the cardiac muscle fibers and in part surrounding branches of the coronary vessels. In many places, the fibrotic areas were very poor in nuclei and showed only a few spindle-shaped cells and an occasional lymphocyte. In other portions, the fibrosis was apparently younger, showing more connective tissue cells and a few blood vessels with indistinctly outlined walls. Other sections revealed typical organization tissue with many newly formed blood vessels, many lymphocytes, endothelial cells and a few polymorphonuclear leukocytes. In some of the hearts in which the mouths of both coronary arteries were found to be encroached on, a progressive fibrosis was noted, with pictures varying from that of early necrosis to that of organization tissue and that of old fibrotic plaques. In a few cases, some of the sections revealed a basic staining of the con-

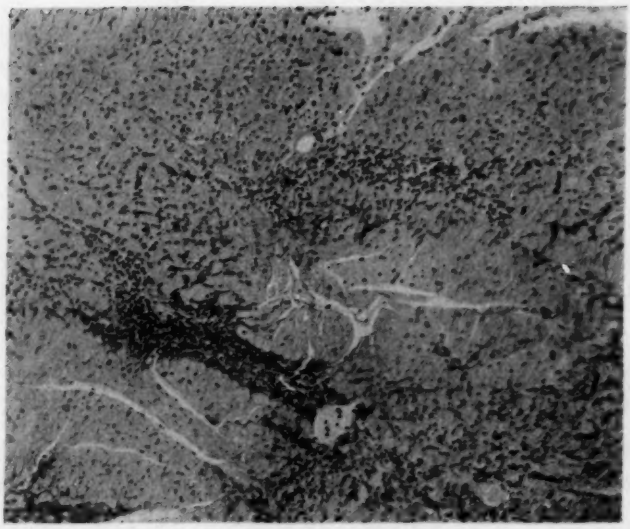

Fig. 1.—Note the polymorphonuclear leukocytic infiltration surrounding an infarcted area of the myocardium (syphilitic occlusion of the mouths of both coronary arteries). Iron-hematoxylin-eosin preparation; × 80.

Fig. 2.—Polymorphonuclear leukocytic infiltration close to an early infarction of the myocardium (syphilitic occlusion of the mouths of both coronary arteries). Iron-hematoxylin-eosin preparation; × 80.

nective tissue and a blue interfibrillar substance that could be interpreted as mucoid degeneration. In these portions a few stellate cells were found. Several cases showed a slight, but distinct, perivascular infiltration of lymphocytes about the branches of the coronary arteries. Typical endarteritis obliterans, however, was not observed. Sometimes a more cellular infiltration was found, pronounced mainly in the interstitial tissue. Lymphocytes, plasma cells, endothelial cells and an occasional polymorphonuclear leukocyte were present. The case described before, that of the 29 year old woman, which grossly showed evidence

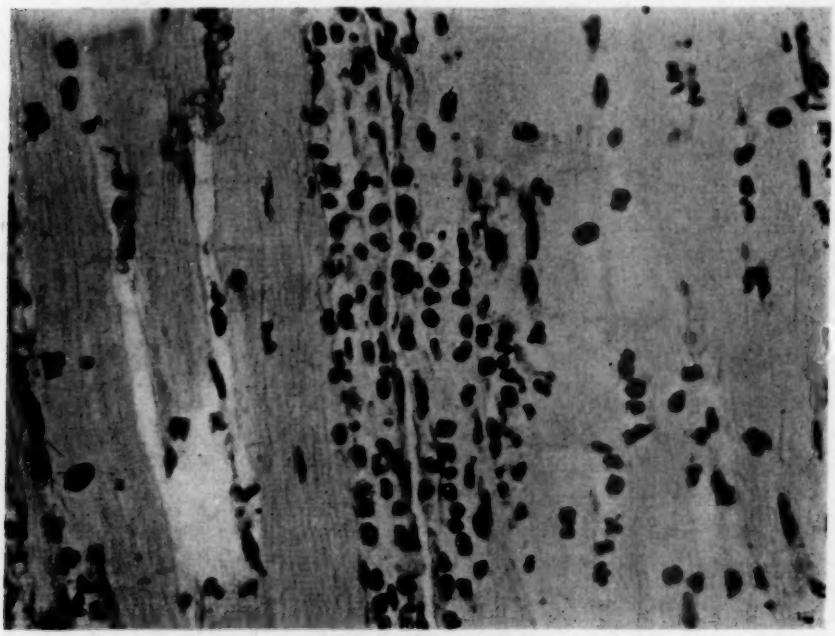

Fig. 3.—Polymorphonuclear leukocytic infiltration surrounding an early infarcted area of the myocardium (syphilitic occlusion of the mouths of both coronary arteries). Iron-hematoxylin-eosin preparation; × 380.

of an infarction of the myocardium, revealed, on section, in addition to the recent necrosis of the muscle fibers, many areas of infiltration by polymorphonuclear leukocytes between which a large number of lymphocytes and endothelial cells were present (figs. 1, 2 and 3). No relationship could be demonstrated between the degree of stenosis of the mouths of the coronary arteries and the changes in the myocardium. In a few cases that showed encroachment on both mouths of the coronaries, the myocardium revealed no changes of note.

With every section of the myocardium in these cases which was stained for spirochetes, tissue from a case of congenital syphilis was

stained simultaneously as control. The control section was examined first, and only when spirochetes were found in this control tissue, were the sections of the myocardium of the cases reported here searched for spirochetes. In none of the sections of the myocardiums of the 130 hearts did the Levaditi or the Warthin-Starry stain reveal typical

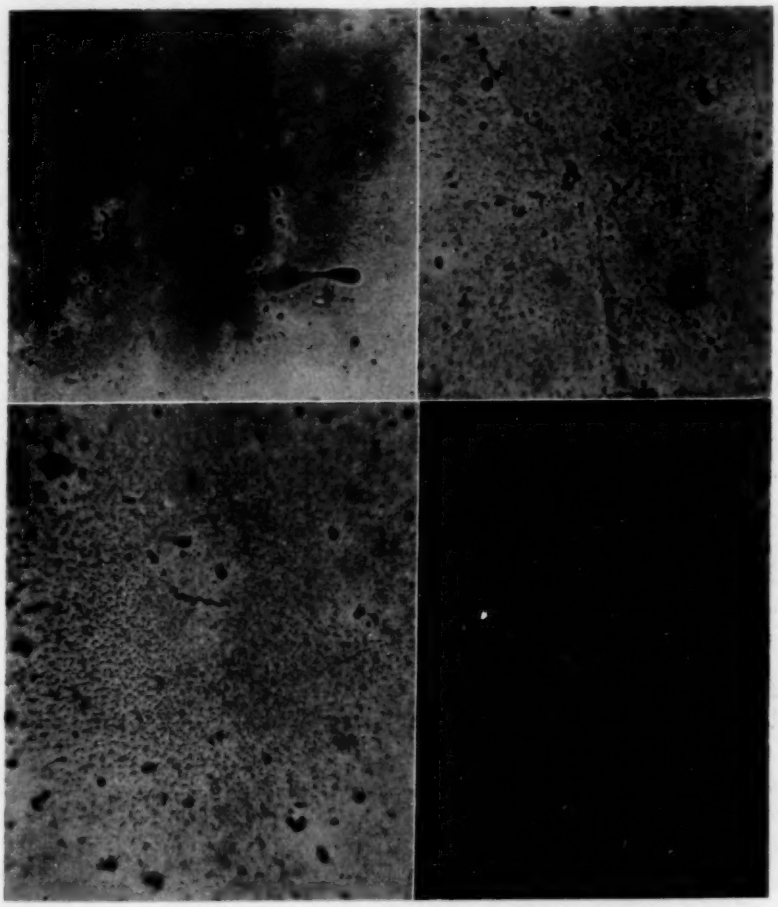

Fig. 4.—Artefacts in coverslip preparation without tissue; Warthin-Starry preparation.

spirochetes, even though in some of the cases sections from 50 different blocks were examined and spirochetes diligently sought.

After this failure to demonstrate typical spirochetes in the sections, it was thought wise to examine coverslips prepared according to the Warthin-Starry method, but without the tissue. In a number of such

slides, it was possible to demonstrate artefacts that somewhat resembled spirochetes. Figure 4 shows some of the artefacts. Similar artefacts were also produced by the use of the Levaditi stain, but much less frequently.

COMMENT

The morphologic changes in the cases on which this study is based were, at least, not characteristic of syphilis. All the changes could be interpreted as the result of coronary sclerosis or as following encroach-

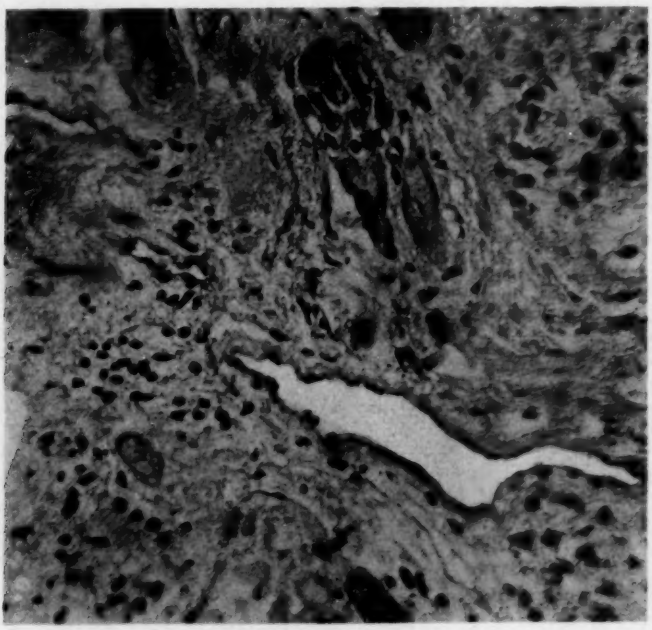

Fig. 5.—The myocardium in a case of marked coronary sclerosis. Note the perivascular infiltration of lymphocytes. Iron-hematoxylin-eosin preparation; × 260.

ment on the mouths of the coronaries. Even though some of the cases showed a perivascular infiltration of lymphocytes in the myocardium, and even though in some of the cases lymphocytes, plasma cells and endothelial cells were found in the interstitial tissue, there were no grounds for believing that these lesions were syphilitic. Similar changes were found in the myocardium in cases of coronary sclerosis, cases which were without a history of syphilis and which at autopsy showed no evidence of syphilis. Figures 5 and 6 show myocardial areas in these control cases. It is of special interest to note that the case that showed a recent infarction of the myocardium revealed histologically

many polymorphonuclear leukocytes in addition to necrosis, lymphocytes and endothelial cells. The presence of polymorphonuclear leukocytes in the myocardium in cases of syphilis has been interpreted by some authors as significant of active syphilis. On the other hand, polymorphonuclear leukocytes were demonstrated by Wearn in infarcted areas of the heart, and are therefore as such not characteristic of syphilis.

SUMMARY AND CONCLUSIONS

A review of so-called chronic syphilitic myocarditis is given and the criteria of syphilitic myocarditis as recorded in the literature are

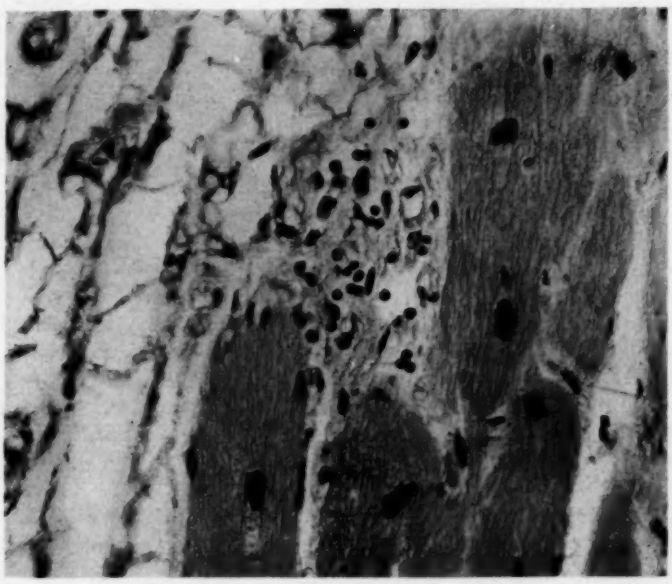

Fig. 6.—Myocardium in a case of coronary sclerosis. Note the perivascular infiltration of lymphocytes. Iron-hematoxylin-eosin preparation; × 380.

discussed. A critical consideration reveals that morphologically the diagnosis of syphilitic myocarditis cannot be made in any of the reviewed cases without the presence of gummas.

In a study of 130 cases of syphilitic aortitis with insufficiency of the aortic valve, the myocardium showed no changes that could be interpreted as syphilitic myocarditis. All the changes found in these hearts might be encountered in other conditions. Spirochetes could not be demonstrated in any of the 130 hearts. Coverslips without tissue, prepared according to the Warthin-Starry method, revealed artefacts that resembled spirochetes.

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THE ETIOLOGY OF CANCER

IV. CANCER METABOLISM *

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OMAHA

OXIDATION

That cancer might be the result of impairment of oxidative metabolism was suggested by van den Corput in 1883. The idea was elaborated in some detail by Wakefield in 1902; according to his view, the normally acid products of cellular oxidation require a certain degree of alkalinity for their neutralization, and in the absence of this, catabolic changes are retarded. Cancer, he believed, occurred in circumstances in which there was this retardation of catabolism either by local acidosis or by other causes of reduced oxidation, while conditions were still suited to maintain anabolic activity. In a study of the metabolism of cancer published in 1914, Braunstein was unable to find any constant change of oxidative activity in malignant tissues, and Burrows in 1917 found that availabilty of oxygen had little influence on growing tissues, as chick embryonic tissues were capable of growth in atmospheres ranging from pure oxygen to partial pressure of that gas as low as 45.6 mm. of mercury. With pure oxygen, however, there was some acceleration of the rate of growth—a finding that was confirmed in 1920 by Russell and Gye. More definite indications of an association between oxidative metabolism and cancerous growth were reported in that year by Russell and Woglom, who found that in mouse tissues there was in general a rise of respiratory quotient with increasing rate of growth. In the case of slowly growing tumors Russell later found that there was a low respiratory quotient, which, however, in the presence of a direct supply of dextrose became greater than unity.

Studies of the oxidative ferments of cancerous tissues have given somewhat variant results. Hugonenq and Paviot found in the rapidly growing portion of malignant tumors an increased content of these ferments; Harden and MacFayden were able to find in the juices expressed from human cancers, catalases and oxidases; Buxton found peroxidase present as in normal tissues, but could not definitely establish the presence of oxidase. As concerns catalase more specifically, Jolles

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and Oppenheim found that in the blood of cancerous subjects this might at times be normal, at times greatly reduced in amount; Blumenthal and Brahn, that there was less of this ferment in cancerous than in normal livers, particularly as regards actually cancerous nodules, but with associated diminution in the uninvolved portions of these livers as well. Rosenthal, in mice with subcutaneous tumor implants, could not find any abnormality in hepatic catalase, but in animals in which implantation had been made intraperitoneally, it was markedly reduced both in the blood and in the liver. Rhodenburg could find no constant variations in the blood catalase content in cancerous patients—a finding which has been confirmed recently by Olchowskaja and Bestschinskaja. Lewis and Corsman found in the Rous sarcoma very little catalase, but more in rat tumors; a result which they believed might have been due to associated infection. Gröbly would attach much importance to the oxidative ferments of purine base metabolism, in part because of the nucleoprotein metabolism concerned with cellular growth, in part because of their abundance in certain internal secretory organs, and he regarded a pathologic increase in nucleoprotein metabolism as a constitutional anomaly predisposing to cancer. Investigation of these particular ferments has shown no very striking results. Saiki found in cancers both guanase and xanthinoxidase, with an absence of adenase, and Wells and Long found that in purine base content and in that of purine enzymes cancerous tissues differed little from normal ones. That, however, there may be abnormal fermentative activity in cancer with respect to oxidative activity was shown by Alsberg, who found in a metastatic melanosarcoma a ferment capable of oxidizing pyrocatechin into a melanin-like substance. Less specific investigation of the oxidative ferments of malignant tissues, as made by Drew with reference to the reduction of methylene blue, showed a lessened rate of this reduction, indicating a low avidity of tumor tissues for oxygen, a finding which has been confirmed by Heinlein and by Menten, and which is in direct contrast to the results of Shearer's study of the fertilized sea-urchin's egg, in which he found that immediately after fertilization there was a greatly increased rate of oxygen consumption. Using microchemical methods, Roskin found in tumor cells three different relations to oxidation—that of cells showing little variation from normal, in which the nucleus is the site of oxidative, the cytoplasm of reducing, activity; that of other cells with this relationship reversed, and that of a third type of cells in which oxidation occurs throughout both nucleus and cytoplasm.

Neuschloss found that the serum of cancerous individuals greatly increases the respiratory activity of normal cells; as concerns their tissues, he found that there is a general diminution of reducing power,

to which rule the spleen is an exception; but the reducing power of malignant tissues was not affected, as was that of normal tissues, by the addition of hydrocyanic acid, and it was not increased to the same degree as with normal tissues by the addition of iron salts. Händel and Tadenuma also found marked impairment of cellular oxidation in rats with large malignant tumors. Remond, Sendrail and LaSalle found that the basal metabolism of tarred rabbits increases during the precancerous stages, but diminishes with the onset of actual cancer. While they would associate these changes with hypothyroidism from intoxication, they could even more probably result from general alteration of cellular oxidative activity. Sehrt observed that differences in the oxygen-combining power of blood, as detected by the addition of alpha-naphthol and dimethylparaphenylendiamine bases with and without the addition of potassium ferricyanide, are considerably less in cancerous blood than in normal. That the relations of tumor growth to oxidative metabolism would appear to be of secondary importance is indicated by the work of Wind, who found that Rous tumor cells would grow freely for as long as 48 hours in almost strictly anaerobic conditions if dextrose was present, whereas no growth took place even in aerobic conditions if it was lacking. Fischer and Buch Anderson found that cultured tumor cells could be killed by excessive oxygen tensions, malignant (sarcoma) cells in mixed cultures succumbing more rapidly than the normal connective tissue elements. With the reversed relation of reduced oxygen tension, Mottram found that sarcoma cells were capable of activity at much lower oxygen concentrations than normal cells, short of actually complete anaerobiosis. These results were confirmed by Wright, who found that while chicken heart myoblasts required a minimum oxygen tension of 12 mm. of mercury to permit cell division, the Jensen rat sarcoma could proliferate at 6 mm., and a mouse carcinoma at 3 mm. of mercury pressure.

Barry, Bunbury and Kennaway investigated the possibility of accounting for the cancerogenic power of arsenic on the basis of interference with the process of cellular oxidation; while the salts of arsenious acid were found to interfere with a number of reduction systems, this was not true of arsenates, which have been shown by experiment to be equally effective in inducing malignant growth, and they were forced to conclude that the effects of arsenic compounds in this direction were indirect, through the accumulation of organic compounds absent in the normal cell.

Among the more detailed theories that would attempt to explain the phenomena of cancer by abnormalities of intracellular oxidation is that of Bristol, who believed that the essential change is one of ferment excess, especially excess of oxidases, possibly brought to the site

of the new growth by migrant leukocytes, with resultant speeding up of biochemical reactions; the formation of lactic acid in malignant tumors would be explained by local oxygen starvation caused by the increased demand of the neoplastic cells for oxygen. Another of the theories of this character is that of Stoltzenberg and Stoltzenberg-Bergius based in part on the work of Saxl, who had found that in advanced cases of cancer, at least, there was an intermediate disturbance of protein metabolism, with excessive formation and elimination of incompletely oxidized protein waste products. In these circumstances, according to Stoltzenberg, the weakening of oxidative ferment action would be most manifest at the expense of the aromatic amino-acid compounds, and there would be an accumulation of these, which in general are cancerogenic, as is the case with indol and skatol, and less certainly with quinone. Burrows regarded his theoretical growth-stimulating substance, archusia, as an oxidation product, which in circumstances of stagnated circulation is accumulated in tissues destined to become neoplastic. Handley would see in diminished oxygen supply one of the factors that induce cancer, along with hypernutrition and reduction of hormones, all results of lymphatic stasis. Perhaps the most carefully elaborated of these theories is that of Brach, who differentiates between active and inactive oxygen, the former of which alone is capable of supplying cellular necessities. Activation is accomplished by means of hormones and vitamins, and probably also by certain mineral substances of vegetable origin. Insufficiency of these, or diminution of "activated" oxygen by the accumulation within cells of abnormal reducing substances, forces the cell to rely principally on the aerobic cleavage of dextrose for its principal source of energy. The lactic acid formed by this may lead to hydrolysis of proteins, or possibly to lowering of surface tension, with increase of cellular nutrition and further suppression of oxidation; in these circumstances, in the presence of an added element of irritation, there is an absence of normal inflammatory reaction and the products of cellular disintegration contribute to the material at hand for the growth of the cancer cells.

REACTION

Among the earlier works that furnished the suggestion that the phenomena of malignant growth might rest in part at least on a basis of altered reaction was that of Jacques Loeb, who found that the immersion of the larvae of Arbacia in weakly alkaline solutions accelerated their development and growth, while immersion in weak acids had the reverse effect—results which he explained on the grounds of acceleration of oxidation in the one case, and retardation in the other. In 1905 Moore, Alexander, Kelly and Roaf reported that in cases of malignant disease, regardless of the location of the lesion, there was a reduction of total

gastric acidity, which they interpreted as being the result of diminished hydrogen ion concentration in the blood, with relative increase of hydroxyl ions, giving a condition of relative alkalinity similar to that in which Loeb had observed acceleration of growth. The same workers reported effects similar to those previously observed by Loeb, in studies made on the fertilized eggs of the sea-urchin, and found that with the addition of small amounts of alkali to the medium in which the eggs were immersed, there followed not only an increased rate of growth, but also the appearance of irregularities in the size and shape of the cells, and at times atypical mitotic figures. Their findings in regard to gastric secretion were not confirmed by Copeman and Hake, who found in the stomachs of cancerous mice an increased acidity. The theory of alkalinity as a factor in the induction of cancer was again advanced by Hertzler, who regarded the disease as originating at sites where there was a combination of irritation and exposure to alkaline secretion. McClendon and Mitchell, reasoning again from the changes induced in the developing sea-urchin's egg by alkalinization, regarded increased cellular permeability as an important adjunct to the process, as with such a change incident to cellular fertilization or parthenogenetic development, the increased production of carboxyl radicals and their free escape from the cells would lead to their substitution by hydroxyl, with increased oxidative metabolism as the result of the latter change. Menten, investigating the matter of possible alkalinity in cancer as this might be reflected in the blood, confirmed Moore and his co-workers in reporting that in cancer there was a relative alkalosis, though this was not specific to the disease.

The observation of Benedict that between 75 and 80 per cent of gastro-intestinal cancers occurred at sites of the elimination of acid could be interpreted as in accordance with these views, although it was to the acid character of the surface reaction that his attention was directed. Ahlgren, in a study of tumor metabolism published in 1923, again emphasized the importance of reaction as a determining element in respiration, but hesitated to draw any conclusions, the more particularly since he found some evidence that suggested that actually there was a lesser intensity of respiratory activity in tumor tissues than in general in normal tissues, his basis for comparison being muscle.

Theoretical considerations of the importance of alkalosis in the development of cancer have in more recent years been advanced by Rohdenburg, who would postulate the process as one of repeated and successive cellular destruction, with alkalinization and lysis of the dead cells; from the accumulation of mineral constituents there results a condition of local hypertonicity, and from this in turn there is an increased influx of body fluids and local overnutrition, with increased and eventu-

ally unrestrained hyperplasia. Laville would ascribe cancer to increased oxidation in association with alkalosis, along with an upsetting of conditions of electrical equilibrium; Willy Meyer postulated alkalosis as one of the elements in his series of precancerous changes. Butts regarded the stimulation to malignant growth as essentially the development of a preponderance of positive ions within the cell—fundamentally a condition of intracellular acidosis. This theory he based on the experiment of connecting the healthy portion of an implanted Flexner-Jobling carcinoma in a rat by wire with a tumor-free litter mate, in which circumstances he observed a flow of current away from the tumor.

In a study of changes in reaction incident to the onset of cancer, Remond, Sendrail and LaSalle found that in tarred rabbits during the precancerous stages there was an actual lowering of the p_H value of the blood plasma, which approached the point of acidosis with the development of actively malignant growth, after a temporary increase of the alkaline reserve coincident with the appearance of cancerous change. Woglom, in a direct study of the reaction of tumor tissues, found that while these were more alkaline in reaction that normal muscle tissue, there was little difference in this respect between them and other normal tissues as they occur in the rat. Harde and Danysz-Michel, and Henri, have found that in transplantable tumors of rats and mice, as well as in growing embryonic tissues, there is an increased acidity, with the hydrogen ion concentration ranging from p_H 5.8 to p_H 6.2. Millet noted a less pronounced acidification, with an average hydrogen ion concentration of p_H 6.78, as compared with a normal of p_H 6.92, and Milone found in spindle cell sarcoma of the rat transplanted into the eyeball a hydrogen ion concentration of less than p_H 6 within 4 or 5 days after the implantation. The reverse finding of increased alkalinity in animal tumors was reported by Goldfeder, who found in a number of neoplasms—Rous sarcoma and mouse carcinoma and sarcoma, hydrogen ion concentrations ranging from p_H 7.5 to p_H 7.7. However, the alkalinity was greatest in necrotic portions of the tumors; as might be expected, it was decreased by abundant supplies of dextrose. Reding, and Bischoff, Long and Hill, and Chambers and Kleinschmidt, found a relative alkalosis of the plasma in most cases of cancer—a condition that, according to the first-named, precedes the onset of the disease and is present to some degree in relatives of cancerous subjects, from which he deduces its importance not only in cancerous predisposition, but in the hereditary character of this as well. Chambers and Kleinschmidt, on the other hand, could find no evidence that the change bore a primary relationship to cancer, and Reding's conclusion was disputed by Sannie, who considered that the methods of Reding and his

co-workers were not sufficiently accurate to warrant conclusions so absolute; his interpretation of the alkalosis of cancer is that it is a compensatory mechanism.

As concerns conditions favoring growth of cancer cells in vitro, Fischer found that the cells are more sensitive to acidification than are normal fibroblasts, and that their growth ceases at p_H 5.9; on the other hand, they resisted alkalinization better than did normal cells. In connection with Gye's work on the filtrate of Rous sarcoma, Harde suggested that the former's results might be due in part to acidification of the filtrate during the course of the experiments, as Harde had found that acidification up to a certain point actually enhanced the virulence of the Rous filtrate. This finding could be confirmed only in part by Baker and McIntosh, who found that the effect of acidification was a complicated one, dependent in part on the presence of proteolytic ferments; in ordinary circumstances they observed an effect opposite to that of Harde.

INORGANIC CONSTITUENTS

In 1903 Braithwaite advanced the theory that an excessive intake of common salt was the cause of cancer, as in England, at least, the disease was relatively rare among the Jews, who notoriously do not consume salted pork products, and as, in his opinion, the decreased elimination of hydrochloric acid and the alleged increase of blood chlorides pointed, however obscurely, to such a relationship. Sykes promptly called attention to the fact that in normal persons excessive intake of salt is followed by its prompt elimination, and noted that in sailors of older days, forced to live in large part on salted meats, there was no noticeably greater incidence of cancer than among their shore-faring fellows; Urquhart cited several reasons why the theory should not receive any great consideration—the fact that even when salt meats played a larger part in daily diet than they do now, cancer was no more frequent, and that during a period of greatly reduced consumption of salt in Scotland, occasioned by excessive taxation, there was no reduction in the incidence of cancer. The theory was again revived by Robinson in 1917, on the basis of experiments by Carrel, which showed that tissue growth in vitro was accelerated by increased concentration of salt in the medium. Robinson's detailed theory was far from clear; it embraced the idea of a substitution of potassium by sodium salts, the liberation of sodium by the formation of hydrochloric acid, and the oxidation of the metallic element—a conception which it is hard to reconcile with the facts of everyday chemistry. The directly reversed view was embraced in the theory of Packard, that cancer is due to the artificial demineralization of present day food, an idea based on the alleged rarity of cancer in primitive peoples.

The idea advocated by Scherk in 1904, that cancer may result from imbalance of the inorganic elements of the body, has deservedly received more careful consideration. Bristol in 1913 suggested that the development of cancer occurred in a series of stages, the first of which was one of cellular degeneration from local causes, the second one of increased affinity of the tissues for certain inorganic salts, and the third a resultant disturbance of chemical equilibrium, which affected adjacent cells, possibly by increasing their permeability, and stimulated them to excessive and even unrestrained growth. Among the later advocates of the importance of salt imbalance as a cause of cancer, different views have been taken of its character. Dubard regarded it as one of substitution of calcium salts for those of magnesium, with a possible indirect action through the nervous system. Willy Meyer very recently regarded the imbalance as essentially one of replacement of calcium by potassium salts, brought about by a variety of mechanisms —hormonal disturbances, aberrations of the normal relations between sympathetic and parasympathetic nervous systems, etc. In these circumstances there are an alteration of cellular permeability and an alkalosis that permits cells stimulated by locally formed necrohormones to assume the property of unrestricted growth. Much the same view was taken by McDonald, who regarded the change in salts as one of substitution of the alkaline metals for the alkaline earths, with ensuing greatly increased cellular permeability and an alkalosis that in unicellular organisms is followed by increased division. Letulle and Vinay suggested that the greater death rate from cancer in northern France is due to the high content in the dietary of the inhabitants of that region of potassium salts—a result of the intensive use there of potassium fertilizers. De Raadt has rather recently reported the alleged production of cancer in four of ten mice by a diet rich in potassium salts, which in addition was of highly basic character.

Beebe in 1904 and 1905 found that in the degenerated portions of tumors there is about ten times the content of calcium salts as in the rapidly growing portions—a relationship that was reversed in progressing tumors; this finding was confirmed by Clowes and Frisbie, who found that the most rapidly growing and successfully transferable tumors were those with a ratio of potassium to calcium of from 2:1 to 3:2. Cattley in 1907 investigated the occurrence of potassium salts in rapidly growing tumors by microchemical staining reactions, and found that while in malignant tumors the intracellular distribution of these salts did not differ appreciably from that of normal cells, there was a superabundance of them in the more actively growing portions. Wolff, and Rohdenburg and Krehbiel, in 1923 and 1924, respectively, reported likewise an excess of potassium salts in actively progressing transplantable tumors, in the latter case with variations within the

scope of a single such tumor. Goldzieher and Rosenthal in a study of the effects of salts on tumor growth in 1913 reported that on the treatment of animals in which tumors had been implanted with potassium salts there was an increased rate of growth of the tumors, and restraint of this after the administration of calcium salts—a result which was confirmed by in vitro experiments of Cramer in 1918, who found that while the immersion of cells of transplantable mouse tumor in isotonic solutions of sodium salts was without appreciable effect, their immersion in calcium salts was followed by marked diminution of growth; the effect was transitory and was accompanied by loss of water by the cells and an appearance of increased density. Lazarus-Barlow in 1922 attempted to increase the susceptibility of rats to spontaneous development of tumors by feeding them with potassium phosphate, as well as by exposure to the x-rays, after which he attempted to induce tumors by the insertion of radium, with at most inconclusive results. Händel in 1923 again confirmed the results that had been previously reported concerning the effects of the administration of potassium and calcium salts, in that he found that animals fed with the former were not only more susceptible to implantation of tumors, but that in them the tumors progressed more rapidly, while in those fed with calcium salts the reverse was true, though only to a limited degree. He found that restriction of sodium chloride in the diet had no apparent effect, nor was there any after the feeding of phosphates. In contrast to these findings, Herly was unable to observe any differences in the behavior of malignant tissues after immersing them in physiologic solution of sodium chloride, Ringer's solution and Locke's solution. Packard found some evidence that the effects of potassium and calcium salts may be produced in part by other members of their respective chemical groups, in that with Paramecium the rate of division may be regulated, at least in part, by the relative proportions of sodium salts to those of calcium, although with too great disproportions there was retardation of division.

A suggestion of the mode of action of salts in influencing the rate of growth of tissues is furnished by the work of Magath, who found that imbibition by cancer cells after immersion in weak acids is greatly reduced after their preliminary treatment with calcium chloride solution—a phenomenon which is also shown by embryonic tissues, and which would appear to indicate the action as being an effect of alteration of cellular permeability. Lasnitzki would trace the inception of malignant growth to increased growth function caused by colloidal swelling produced by increased intracellular content of potassium salts, which he regards as having the added effect of favoring the hydrolytic cleavage of dextrose to lactic acid.

Delbet would see a relative deficiency of magnesium salts as an important factor in the genesis of cancer. In support of this view he adduced clinical evidence of the subsidence of precancerous lesions after the administration of magnesium salts, and, in guinea-pigs, the failure to develop of cancer-like lesions of the gallbladder after the insertion there of solid bodies, as in Kazama's experiments. Also, in a limited number of rabbits, he observed lack of development of hypertrophic lesions or of cancer after the application of coal tar. With implanted tumors in mice, he reported considerably diminished progress of these tumors under the administration of magnesium salts. In addition, he alleged that the geographic distribution of cancerous and noncancerous areas is associated with the relative absence or abundance of magnesium in the terrain, and stated that in brewery employes, cancer is exceedingly rare—a fact that he would explain by the high magnesium content of most beers. Just why cancer should be not particularly infrequent in countries with high beer consumption is not explained. Finally, he suggested that the alleged increase in the mortality from cancer is to be associated with the elimination of magnesium salts in the process of food refinement. Whatever may be thought of Delbet's views, they receive a possible theoretical support, in view of the importance of carbohydrate metabolism in malignant disease, in Canals' finding that with at least certain carbohydrate-splitting ferments—sucrase and invertase—magnesium takes the same relative importance as does manganese in the oxidases.

Studies of the systemic changes in salt content of the body in association with cancer have furnished results that on the whole are of little significance. Rohdenburg and Krehbiel considered that after the parenteral introduction of living cells, regardless of their type, there was a demineralization of the blood and general tissue cells, with concentration of these mineral elements in the actively growing cells. With the death of the cells, the salts were again returned to the general circulation. These changes, as well as those of altered ratios between various salts, they considered as purely secondary. Theis found that in cancerous serum there was a lowering of the calcium content, while that of sodium was normal, and that of potassium was somewhat lowered as a rule; Paolucci reported the occurrence of a slight rise of blood calcium early in cancer, with a subsequent decrease; Krehbiel found that in human cases blood calcium remains within normal limits. As to potassium, Ross found a deficiency of this in cancerous patients, but Ottonello reported a usual, but not constant, increase of serum potassium, although he did not find this change specific for cancer; Robin found in advanced cases of cancer a high ratio of potassium to sodium, with a reduction of calcium salts—changes which he regarded as secondary to the nutritive changes associated with cancerous lesions of bone or liver.

That the changes in salt and water content are not so much peculiarities of tumors as of growing tissues in general has very recently been indicated by Bricker and Lazaris, who found in regenerating tissues the same increased water content and excess of potassium over calcium.

There is a limited number of studies, likewise of no particular significance, in relation to other inorganic constitutents of cancer cells. Tracy in 1905 could find no differences between tumor and normal cells as regards iron content; Medigrecanu, studying the presence of manganese salts in tumors because of the importance of these in relation to oxidase activity in vegetable tissues, could find no increase of these. Taverne, in a case of human cancer, found the content of copper salts reduced below that of normal tissues, and no particular differences in zinc salt content. Robin found that cancerous tissues have the power to fix to a slight degree silica, chalk and magnesia given in the food.

NITROGEN METABOLISM

Although much work has been devoted to the study of the nitrogen metabolism of malignant tumors, this has failed to reveal any characteristics in regard to which they are peculiar. Unquestionably many of them show increased disintegration of nitrogen compounds, as was pointed out by Müller in 1889; while in 1895 Nepveu reported finding in such tumors indol and indican, as further evidence of drastic protein cleavage. Petry in 1899 found that in cancers there was a relatively low proportion of albuminous nitrogen, although there was no constancy in the proportions of albumins to globulins; nucleoproteins, he found, were increased, and a general increase of noncoagulable nitrogen he ascribed to an increase of proteolysis beyond what occurs in normal tissues. Further support for the conception of increased autolytic activity in cancerous growth was afforded by the work of Emerson, who in 1902 reported the presence, in gastric cancers, of ferments that carried protein digestion beyond the albumose stage. Glaessner in 1903 reported the regular finding of tryptophan in cancerous stomachs, and its formation if cancerous tissue was added to normal gastric juice. However, he found that it might occur occasionally in other circumstances, as in the hyperacidity of gastric ulcer, and Erdmann in the same year found that while tryptophan was present with some regularity in cases of pyloric cancer, it was occasionally absent in that condition, and was likely to occur in almost any condition of gastric stagnation. Later findings of abnormal amino-acids in cancerous stomachs are those of Fischer in 1908, who identified tyrosine, leucine, arginine and lysine there. In 1909 Neubauer and Fischer announced finding in such cases a peptone-splitting ferment to the presence of which they attached diagnostic import, a conclusion that was disputed by Kutt-

ner and Pulvermacher, who found that such ferments were not constantly present in cancer and were occasionally present in normal stomachs. An increased content of amino-acids in cases of gastric cancer has also been reported by Barlocco. As concerns abnormal ferment activity in cancers other than those of the stomach, Blumenthal in 1905, after having previously reported his inability to find anything specific in the metabolism of cancer, stated that he had found in malignant tumors abnormal heterolytic ferments, and Neuberg in the following year announced similar results. In 1907 von Leyden and Bergell, on the basis of altered reactions of tumor tissues to various proteolytic ferments, reached the conclusion that the unrestricted growth of cancer was based on the lack in the host of certain hydrolytic ferments—that it was due fundamentally to a local aberration of protein synthesis, without however implying the necessity of the formation of any specific albuminous substances. Bergell and Dörpinghaus found, in an attempt to determine the amino-acids of cancer tissues, a high content of both mono-amino and di-amino acids, with a strikingly low content of leucine, and Bergell reported that cancerous tissues showed an unusually high content of di-amino acids, which he found constituted 40 per cent of the total nitrogen of both human and mouse tumors, as compared with a normal proportion of about 30 per cent. In sarcomas, however, he found that they amounted to only about 24 per cent. These findings of a disproportion of amino-acids Beebe could not confirm.

Hofbauer advanced a theory of cancerous growth on the basis of abnormal protein cleavage, with the view that infiltrative growth could be explained by the inability of delimiting connective tissue to withstand attack by abnormal ferments, possibly because of a deficiency on its part of antiferments. Westenhoeffer considered the cancer cell as one that had undergone such extreme reversion and modification of ferment activity as to constitute a new, essentially foreign cell. In freshly excised and nonulcerated cancers of the breast he found a content of amino-acids and albumoses absent in normal tissues, and with this evidence of increased proteolytic power on the part of cancer, he based his surmise as to the inability of the stroma to resist these ferments. Rülf in 1909 also believed that explanation of the aberrations of cancerous behavior was to be sought in differences already demonstrated in the albuminous metabolism of cancer cells.

In contrast to those findings, which indicate definite, and possibly specific, variations of protein metabolism in cancer, there is a large amount of work that indicates that the changes found are inconstant and of no general significance. Petry in 1902 found that the products of autolysis of cancer were the same as with normal tissues. Braunstein in 1904 was unable to find any constant peculiarities in the meta-

bolic activities of cancerous tissues; no demonstrable differences were apparent in chlorine metabolism, while with 3 of the cancers studied by him there was an increase of nitrogen output. Beebe found evidence that the abnormal proteolysis frequently shown in tumors was due to circulatory impairment rather than to any fundamental variation in metabolic activity, and Buxton and Shaffer found that the intracellular ferments of tumors were closely similar to those of normal tissues, although at times there might be considerable quantitative differences. Wolff, in an attempt to determine the extent of proteolysis in the living cancerous individual, investigated the appearance of cytolytic products in the urine, with completely negative results. Labbe and Mouzaffer in a similar study observed urinary output of amino-acids regularly only in circumstances of involvement of the liver. Wolff also reported finding the proportional distribution of protein compounds in cancer directly the reverse of that reported earlier by Petry, in that his determinations showed a relative increase of the albumin fraction over the globulins. Such differences, however, as he was able to establish were at most quantitative and inconstant. The report by Brieger and Trebing, that there is an increase of antitryptic power in the serum in cases of cancer, might have lent support to the explanations of cancer on the basis of abnormal protein metabolism, were it not that they found this change a phenomena of cachexia rather than of cancer itself. Further evidence that the proteolytic abnormalities found with cancer were not specific was afforded by the work of Hess and Saxl, who could find no evidence of increased autolysis or heterolysis in malignant tumors, and that of Abderhalden, Koelker, Medigrecanu and Rona, who in detailed studies of the proteolytic ferments of tumors found quantitative, but not qualitative, differences from those of normal tissues. The finding by Yoshimoto, later confirmed by Robin, that there were increased powers of autolysis both in cancerous metastases in the liver and in uninvolved portions of that organ, indicated in all probability a quantitative difference of this sort. Kepinow, too, found that mixtures of various organs and cancer tissues failed to show any appreciable alteration of proteolysis. In a later work Abderhalden and Pincussohn found differences in the proteolytic action of malignant tissues, but these were inconstant. Hamburger was unable to distinguish any difference in ereptase activity between cancer and normal tissues, and Weil, in a study of the behavior of cancerous ascitic fluids, could find no abnormalities of ferment action. Lieblein could find no evidence of constant alteration as regards autolysis and heterolysis by cancer tissues. Falk, Noyes and Sugiura, studying the ferment activities of tumor tissues, found for proteases that while there was some heightening of the activity of these, the ferments themselves were without significant differences from those

of normal tissues; nor were they successful in finding in tumor tissues substances that modified ferment activity. Robin again, in 1926, reported the discovery of highly proteolytic ferments in cancer, and Edlbacher and Merz in 1927 announced that in all the tumors studied by them there was a greatly increased capacity for arginine cleavage, but this increased arginase content they found was a feature of growing mammalian tissues in general, as it was present also in granulations, polypoid growths and embryonic tissues; with fowl tissues the findings were either less decided or negative.

Efforts at tracing abnormalities of nitrogen metabolism in cancer through changes in the circulating blood have shown that here, too, changes are inconstant, and so of little significance, quite aside from the probability of their being secondary. Theis and Stone in 1919 found that in general there was a low serum content of nonprotein and urea nitrogen, with uric acid in normal amounts. Robin in 1920 reported that there was an increase of total nitrogen, and Loeper, Thinj and Tonnet that the blood changes indicated a pronounced disturbance of nitrogen metabolism with increase of residual nitrogen and relative decrease of the urea nitrogen of the blood—a change which they ascribed to the ereptic activity of the tumor, with an added effect through involvement of the liver. Killian and Kast reported in 1921 that in 80 per cent of over 100 cases of cancer they found an increase of blood uric acid, and in 60 per cent an increase of urea nitrogen—changes that were almost certainly secondary, as they were constant in general abdominal carcinomatosis, present in 90 per cent of cancers of the lower part of the abdomen and in 50 per cent of cases of gastric cancer, and rarely present with external cancers. Theis in 1921 was unable to find any changes in the quantity of proteins in the blood plasma, while Loeper, Forestier and Tonnet reported an increase of these, and particularly of the globulins. Bucher in 1922 found by physical methods a relative increase of serum globulin—an observation which was confirmed by Kennaway in 1924, who, however, did not find this change constantly; by Galehr in 1925, and by Lewin in 1927. Both Galehr and Lewin ascribed the change to increased cellular destruction, and as further evidence to this effect Kotzareff and Weyl reported in 1923 that in cancerous rabbits there was an increased blood content of amino-acids and amines, to which they assigned a similar origin.

Some allegedly successful efforts have been made to identify specific albuminous constituents of tumors by means of immunization procedures. Loeper, Forestier and Tonnet believed that they could sensitize guinea-pigs to cancer albumins by the injection of the serum of cancerous patients, and Seyderhelm and Lampe reported that the alcoholic extraction of human cancerous material yielded a substance extremely toxic to mice, provided the extraction was conducted at

extremely low temperatures; Mertens likewise was able to secure similar toxic material from tumors; but such findings are of little exact significance in view of the known toxicity of partial protein cleavage products. Very recently Auler, Schlottmann, Rubenow, Meyer and Wolff reported that by means of a carefully prepared antigen they could cause the development of antibodies specific to tumor cells. Still more recently, Ottensooser has reported some not particularly convincing evidence of passive immunization to transplantable mouse carcinoma.

A number of abnormal nitrogenous substances have been reported as present in the urine of cancerous patients, usually with the implication that they are specific for this disease. Obviously if this were true, it would indicate definite alterations of protein metabolism; but they would appear to be products of the destruction of tissue in general rather than of an origin connected with peculiarities of the metabolism of tumor cells. Salkowski in 1905 and subsequent years reported that in such urines he was able to find large amounts of colloidal nitrogen-containing material, precipitable by alcohol or by salts of heavy metals. The substance has been variously identified—as chondroitin-sulphuric acid and compounds of nucleic acid by Sasaki and as a glycopeptide by Ebbeke. The specificity of this urinary abnormality was disproved by Ishioka, who found that material of this character might be present in small amounts only in cancerous persons and at times in large quantities in noncancerous persons; Carforio also found that the change was not specific for cancer; Mancini associated it with increased colloid content of the lymph and blood stream, and Meidner found that it was a manifestation of wasting diseases in general. Another abnormal urinary product was associated with cancer by Salomon and Saxl—a high content of oxyproteinic acids, which showed a wide variation in nitrogen content and a rather definite content of partially oxidized sulphur compounds, although in later publications they stated that these sulphur compounds appeared to have no definite relation to the oxyproteinic acid bodies. The nonspecificity of these changes was pointed out by Pribram, Kaldeck, Kahn and Murachi.

One rather constant abnormality of cancerous tissues in their relation to nitrogen metabolism is the fact that protein substances constitute in them a considerably less proportion of the total weight of the fresh tissue than is true of corresponding normal organs. Cramer and Pringle found that in malignant tumors there is a protein content only about three-fourths that of normal tissues, and that in the growth of tumors there is relatively less nitrogen retention than is necessary for somatic growth. Robin announced similar findings, which he explained in part by the assumption of especially intense proteolytic activity about, but not in, the cancerous tissues, and a resynthesis of

the amino-acids so liberated by the neoplastic tissue. Chisholm found that the disproportion is largely a matter of water content, as he found that in dry tumor tissue there was no constancy in this regard.

Investigations of the relations to cancer of single amino-acids serve to illustrate the inconstant character of unusual features of protein metabolism in this disease. Fränkl observed that while tryptophan was lacking in skin cancers and in melanotic tumors of the skin, it appeared in those conditions in excessive quantities in the urine. But that the abnormality of metabolism that this would indicate is variable, even with the special types of malignant growths considered by Fränkl, was indicated by Fasal, who found tryptophan in increased amounts in some skin cancers; he found it also in metastatic cancers of the liver, but it was lacking in a case of carcinoma of the breast studied by him.

The study of the peculiarities of the growth of malignant tissues in vitro has revealed a number of characteristics in which these differ from their normal prototypes in respect to nitrogenous metabolism. Carrel and Burrows reported in 1911 that the addition of the plasma of another tumor-bearing fowl to cultures of fowl sarcoma had the effect of a marked inhibition of growth, while the plasma of the tumor-bearing host was without this action. The addition of sarcoma extracts caused increased growth not only of the tumor cells, but of normal tissues as well, and surpassed in this respect extracts of embryonic tissues. A similar effect of apparent immunity was shown by the addition to cultures of mouse sarcoma of the plasma from tumor-bearing rats, while the plasma of normal rats permitted free growth, as shown by Lambert and Haynes, who ascribed the inhibitory action to cytotoxins. A familiar finding in work with tissue cultures was that embryonic extracts were almost essential for growth, while the plasma could be substituted by other substances, as for instance calcium in colloidal solution, as was shown by Drew; so apparently the unusual effects of what may be termed immune plasma would not appear to be associated with nutritive and thence metabolic factors. The same statement is also probably true of the inhibitory effects on tissue growth shown by the plasma of aged persons, as shown by Carrel, or by extracts of adult tissues, found by Mottram. On the other hand, the effect of embryonic extract, and also of the extracts of sarcomatous tissues, would appear to be due largely to nutritive elements contained in them, as was found by Baker and Carrel largely in connection with cultures of normal tissues. They found that, as a substitute for these extracts, partially digested proteins from various sources might be used; but while these proteoses sufficed, the substitution of amino-acids did not suffice, for the building up of new cellular protoplasm, although they had the effect of promoting cellular migration. In these respects there was not strict correspondence between normal and neoplastic tissues; with the

partially digested products of casein or egg albumin, the proteoses were suitable for the growth of normal fibroblasts, while for sarcomatous fibroblasts the further addition of glycocoll and of nucleic acid was necessary for rapid growth. On the other hand, partially digested material from liver permitted unlimited growth of sarcoma cells, while it served only temporarily for the growth of normal cells. That different tumors show variant needs in the way of nitrogenous material was shown by Carrel in connection with various tumor strains. Further differences between tumor and normal tissues were shown by Fischer. He found that with a strain of mouse carcinoma the addition of rat serum would suffice for nutriment in the absence of embryonic extract, as would such extract after inactivation at 56 C.—features lacking with normal tissues. A characteristic of malignant growth that Fischer particularly emphasized is the ability of tumor cells to obtain their nutriment from nearby cells with which they come into contact. Zakrewski, in a study of a number of different tumors, found that this ability to destroy and utilize normal cells is not a constant property of malignant cells, and that the liquefaction of the plasma clot that adds so greatly to the difficulties of culture of many malignant tissues also occurs in normal tissues in circumstances of cellular disintegration. It would seem that this feature is accomplished by a similar process in the case of neoplastic tissues, particularly as Zakrewski found the latter unusually sensitive to intoxication by products of cellular decomposition.

In regard to nucleic acid metabolism, the study of cancer has revealed as little in the way of specific changes as with nitrogen metabolism in general. Bang in 1904, as a result of determinations of nucleic acid content in the metastases of a malignant testicular tumor in lymph glands, found in them approximately the same content as that of the invaded tissue, and Beebe made a somewhat similar observation in that while he found nucleohistone absent as a rule in primary tumors, it was present in their metastases to lymph glands, from which he inferred an influence in this respect of surrounding tissues on the processes within the cancer cell. Tracy could find nothing peculiar in the phosphorus content of malignant tumors, but Beebe, in common with Petry and Wolff, found in malignant tumors a relatively high proportion of nucleoproteins. Probably largely from these findings Skerrett inferred that cancerous growth required an especial abundance of nuclear material, which he surmised was obtained from the disintegration of adjacent cells. In 1912 Calkins, Bullock and Rohdenburg reported that toxic substances, presumably belonging among the nucleoproteins, were capable of stimulating to excessive mitosis even relatively fixed tissues—pancreas and liver—with the appearance of changes which they were inclined to regard as precancerous. They advanced

the hypothesis that, as a result of local conditions which might be determined by a variety of causes, products of local autolysis were liberated, which stimulated the division energy of previously latent cells. This is essentially similar to the necrohormone theory as advanced by Ross and again and more recently in connection with postulated changes of more general character by Willy Meyer.

The finding by Ordway and Morris that there is a marked increase in the urinary uric acid output in cancer, might presumably be interpreted like other abnormalities of urinary elimination in cancer, as being brought about by excessive destruction of tissue. Wolter, in a comparison of the phosphorus contents of metastatic cancer of the liver and the adjoining hepatic tissue, found a diminished amount in the former, principally as a result of a reduction in the amounts of lipoid and protein phosphorus; but the latter was increased as compared with normal, noncancerous liver. Enselme found evidence of increase of nucleic phosphorus in malignant tumors, with a content ranging from 28 to 35 mg. per hundred grams of fresh tissue, as compared with from 13 to 21 mg. for corresponding normal organs. Goodman found the presence, in nonulcerating mammary cancers, of considerable quantities of nuclease, with the presence of which he associated the occurrence of cancerous cachexia.

A number of writers have asserted that in association with cancer there is marked alteration of the phosphorus content of the circulating blood. Von Moraczewski reported in 1895 that cancerous persons showed a diminished amount of blood phosphorus, but to no greater degree than occurs in anemia from any cause. In 1921 Gröbly found that in proportion to the number of red cells the phosphoric acid radicals in the blood are considerably increased, a finding that has been confirmed by Vorschütz and Vorschütz, by Zerner and by Wohlfarth, although the last named observer found a similar increase in cachexia from other causes as well. Buckman, Minot, Daland and Weld, however, found that the total phosphorus content of the blood is roughly proportional to the hemoglobin content, and like Wohlfarth are inclined to attach little special significance to this finding in cancer, a view which is shared also by Byrom and Kay.

NECROHORMONES

The idea that malignant growth might be the result of a discharge of growth-stimulating material from dying or degenerating cells has been advanced a number of times. In 1902 Marchand, in a comprehensive discussion of tumor etiology, after discounting the theory of parasitic origin, expressed the belief that in certain conditions of cellular degeneration there are formed toxic materials the accumulation of which lead to a change of cellular behavior, with a liberation of the affected

cells from normal regulatory mechanisms. In 1903 Clowes, although he favored the parasitic theory, acknowledged the possibility of an action of stimulant toxins formed within the cell or at a distance from it. Skerrett in 1907 surmised that the superabundance of nuclear material that he regarded as essential to cancerous growth might find its source in the disintegration of the nuclei of dying cells. In 1910 Stoeber and Wacker observed that on injection into the rabbit's ear of products of protein cleavage—pyridine, indol and skatol—growths were obtained closely resembling human skin cancer, and suggested that malignant tumors might owe their origin to an excess production of similar substances, and their anchoring by the proliferating cells. Bruntz and Spillman, in the following year, considered leukocytic infiltration as leading directly or indirectly to epithelial proliferation, and if this stimulation were sufficiently prolonged, the actively proliferating cells would divert more and more of their energy to this, and ultimately abandon their functional activities and become permanently committed to a process of unlimited growth. In 1911 and 1912 Ross reported observations in which he found that leukocytes, after exposure to extracts of dead and decomposing tissues, underwent rapid division. The substances responsible for this he termed auxetics, and found that, as a rule, they were such as contained the amidine radical. There appeared as well to be a second class of substances, which he termed augmentors, which had the property, as his name for them suggests, of augmenting this growth-stimulating action. He advanced the hypothesis that malignancy is due to the discharge of these substances from necrotic tissue, with a resultant stimulation of the regenerating tissues to unlimited growth. The process could become continuous as a result of the continuing death of some of the cells, serving as a constant supply of the stimulating materials. Rohdenburg and Bullock in 1912 considered that the continued presence of nucleoprotein hydrolysis, if removal failed to take place, could act as a stimulant to continued cellular reproduction, and Calkins, Bullock and Rohdenburg, as stated, found that substances of this sort could stimulate innumerable mitoses even in relatively fixed tissues, such as pancreas and liver, although definitely cancerous growth was not observed by them. Jones and Rous in 1914 found that injury of tissue appeared to be essential to the successful intraperitoneal implantation of mouse tumors; their explanation, however, was that in these circumstances there was greater certainty of provision of stroma for the introduced tumor. In 1916 Calkins announced that extracts of epithelial tissues in general had a growth-stimulating effect on free-living protozoa, but that cancerous tissues had an added lethal effect; he suggested that the stimulating substances might be of autolytic character, and that cancer might be due to their liberation and constant action in a focus of continuous destruction and repair of tissue. Robin in 1919 advanced

the hypothesis that cancerous activity might be the result of intense proteolytic activity around, not in, the malignant focus—the excess of water in and about malignant tissues being his evidence for such activity—and that resynthesis of material set free in this way could serve as the cause of the unrestricted growth. Caspari in 1922 regarded the necrotic portions of tumor tissue as a possible source of nutritive material for surviving tumor cells, and Haberland regarded such substances as definitely in the nature of growth-promoting hormones, which however might be in part of extraneous origin. That radiant energy stimulated excess tissue growth by means of the liberation of necrohormones from irradiated tissues was suggested by Caspari in 1923. In that same year, Drew described his experiments, previously cited here, in which he found that tumor extracts and extracts of autolyzed normal tissues had growth-promoting powers. Crawford in 1924 suggested that the scrotal cancers of cotton-spinners were due only indirectly to the lubricating shale oils, the accumulation of dead and dying epithelial cells in the scrotal folds being the element directly responsible. Eastwood in 1925 considered that cancer originates in circumstances of exclusion of the implicated cells from access of growth-regulating elements of the plasma, without nutritive deficiency, the stimulation to growth being furnished by the autolysis of cells that have succumbed. Essentially the same idea forms part of Willy Meyer's theory of the inception of tumor, as well as of that most recently expressed by Caspari, and Müller would see cancer as caused by necrohormones that are specific in that they are derived from disintegrated tissues of the sort which they stimulate to reproduction.

There can be little question that growth-stimulating materials occur in tumor tissue; Drew's experiments with tissue cultures; those of Erdmann and Haagen, and Burrows, in which the injection of tumor filtrates alone or in conjunction with embryonic tissues determined the inception of new tumors, even though possibly infrequently; those of Eggers, who could observe delimited overgrowth of regenerating connective tissue after similar injections—all indicate such stimulating action. As to the nature of the stimulating substances, they are usually regarded as products of protein cleavage; Wright found that similar substances obtained from embryonic tissues could pass through collodion filters impermeable to proteins, and Phillipson was of the opinion that complexes containing the pyrrole ring were responsible for stimulation of this type, not only as derived from disintegration of tissue, but as the responsible agent as well of most known cancerogenic chemical substances.

The principal objections to the several theories of necrohormones as the agents responsible for cancerous growth are based largely on indirect evidence. If such substances played the important part assigned to them

in some of the theories, it would not be unreasonable to expect cancer to occur more regularly in circumstances of continued destruction of tissue accompanied by regeneration, as for instance in lupus, which, while precancerous with some frequency, is not so at all regularly. Also, the limitation of malignant growth to cells of a single origin, which is so usual that exceptions are invariably considered deserving of special report, would appear incompatible with these theories, unless one accepts Müller's idea of specific necrohormones, which again is scarcely in accord with what would appear to be relatively simple chemical composition.

FAT AND LIPOID METABOLISM

With regard to the metabolism in tumors of fat in the strict sense of that word, there does not appear to be any constant or significant alteration. In 1906 Dunin-Karwicka, in the course of a general study of fat metabolism, found that vital synthesis of fat could occur only in living and nucleated cells, in circumstances which determined its deposition in droplet form, and established the presence of such fat in living tumor cells. Beatson in 1911, as a result of studies of tumor fat made by Duncan, in which he found a gradual increase in iodine value with advancing age, and an especially high value in cases of cancer, was inclined to attach special and possibly etiologic significance to the increase in unsaturated fatty acids, and while Dominici corroborated this work in the same year to the extent of finding fats present in all the tumors he studied, and finding them different from normal fats, Wacker, in the following year, was quite unable to confirm Duncan's work and the surmises of Beatson. Wacker found that the change in cancerous fat was one of increase of unsaponifiable material, including cholesterol—a change which was not specific for cancer, as it occurred as well in a number of wasting diseases. More recent work by Currie is in partial agreement with that of Duncan, since he found in 1922 that the fat of malignant tissues shows marked variations in iodine value, the local fat of carcinomas showing the greatest proportion of unsaturated fatty acids, while that of sarcomas and chronic inflammatory lesions had a value intermediate between those of normal tissues and carcinomas. In 1914 Bullock and Cramer reported that while variations from normal fats and lipoidal substances in general were frequent in the transplantable tumors of rats and mice, these differences were not constant, and involved even different strains of tumor cells from the same parent tissue. That fats could act as chronic irritants, and so possibly display cancerogenic action, was shown by Yutaka San in 1916 and 1917 and by Kon in 1917, who found that in rabbits and rats after long feeding with hydrous wool fat there was deposition of lipoidal material in the wall of the stomach, which was associated

with papillomatous or adenomatous overgrowth of the epithelium of the mucosa. Lee, Fukuda and Kinoshita in 1924 reported that the feeding of hydrous wool fat to tarred rabbits hastened the development of tumors and accelerated their growth after malignancy was initiated, while with mice Kashiwagi, Fukuda and Owaga found that the administration of hydrous wool fat so greatly shortened their lives as to reduce greatly the incidence of tumor.

A series of studies on the lipatic ferments of tumor tissue made by Noyes, Sugiura and Falk gave results that indicated marked similarity in the lipases of a number of rat tumors, but these differed much from the ferments of mouse tumors, and both were different from the lipases of the Rous sarcoma. As regards tumors of man, two main types of lipases were found, one with reactions resembling those of the lipases of the Flexner-Jobling rat carcinoma, the other quite different. Even in tissues of common derivation—uterine fibroids—two types of lipatic activity were found.

With the lipoids, as distinct from fats, findings of more significance have been obtained. Bossart in 1902 found that in undegenerated malignant tumors there was a considerable amount of lecithin, but little of true fat, the latter appearing in quantity in circumstances of degeneration and necrosis. Werner in 1905 was inclined to ascribe the effects of the x-rays and radium to their action on lecithin, in rendering this more susceptible to ferment cleavage. Reinke in 1908, from studies conducted on the brain of the larval salamander, in which he observed atypical growth after the action of ether, ascribed this to injury of intracellular lipoids, which he regarded as exercising a physicochemical restraint, interference with which occasioned unregulated growth. In similar experiments with embryonic tissues implanted in rats, he obtained some evidence of increased, but not malignant, growth, as was also the case when saponin was used instead of ether. Meyer in 1909 presented an elaboration of Reinke's idea; he regarded cellular structure as essentially an emulsion of colloidal proteins and lipoids of such nature as to prevent homogeneity and free chemical activity, with the lipoids forming a protective membrane on the cell surfaces. Other views implicating the lipoids in the genesis of malignant growth are those of McDowell, who considered them as active maintainers of anabolic activity, with malignancy the result of their excessive accumulation. Some experimental support for this conception was furnished by the work of Rosenheim, who found that cholesterol hindered to a great degree the acceleration of fat-splitting effects by bile salts and similarly acting substances, and by that of Wacker and Schminke, with their finding that in general such substances as cause epithelial hypertrophy are lipoidal or lipoid-soluble. Robertson and Burnett, and Sweet, Corson-White and Saxon, found in 1915 that

the administration of cholesterol to animals in which tumors had been implanted facilitated the growth of the tumors and their tendency toward metastasis, and Rondoni has recently reported that the injection of cholesterol greatly enhances the growth of tumors in mice, while that of lecithin restrained it. Luden in 1916 described certain associations between cholesterol metabolism and malignant growth. Not only did she allege a considerable increase of blood cholesterol in malignant disease—a feature that she associated with sex gland function, thereby linking together the increased incidence of cancer at the period of diminishing activity of these glands and cholesterol metabolism; but she postulated further effects in that excessive blood cholesterol was regarded as weakening the defensive mechanism of the lymphoid tissues and promoting cellular multiplication as well. Luden's report of excessive blood cholesterol in malignant conditions has received no very general confirmation. Roffo and Burgheim agreed with Luden, the former finding increase of cholesterol in the serum of patients with cancer, and even to a greater degree, in the tumor itself. Roffo also reported that he had tested for cholesterol content the tumor-afferent and the tumor-efferent blood in fowls, rats and man and found evidence of marked diminution during passage through the tumor, amounting to from 15 to 69 per cent—indications, he believed, of its utilization or destruction there. Denis, however, found that in almost every case studied by him the blood cholesterol was within, and sometimes even below, normal limits. Currie found it slightly deficient, and Mattick and Buchwald found that reduction of plasma cholesterol occurred as a rule. Jacobs reported that after irradiation there was an increase of free cholesterol within the tumor cells. Bolaffi found a pronounced increase of combined cholesterol in tumor-bearing mice, with particular concentration in the tumor itself; but he found little difference in the cholesterol content of the blood of normal and tumor-bearing animals.

In addition to the enhancement of tumor growth which has been reported to follow the administration of cholesterol, is that found by Robertson and Burnett to follow the administration of tethelin, a lipoidal substance isolated from the anterior portion of the pituitary gland. Erdmann found that the injection of this into embryonic tissues implanted in fowls caused increased, though still delimited, growth.

There are a number of experiments in which attempts were made to ascertain more or less directly the action of lipoidal substances on growing tissues. Borst reported that in a certain number of rabbits to the diet of which cholesterol was added, there was an enhancement of the local inflammatory reactions and the cancerogenic effects of the application of tar. Remond, Sendrail and LaSalle observed that while the cholesterol content of the blood of tarred rabbits is usually increased during the precancerous stages, there is usually an abrupt

fall with the onset of actual malignancy, which is followed by a gradual return to normal levels. Shimoda, on the other hand, found that growth of tar cancer is slow in rabbits with a normal blood cholesterol content, and that there is a definite relationship between cholesteremia and rapid growth of tumor. With tissue cultures, Baker and Carrel found that a large element in the growth-inhibiting effect of the serum of aged persons is due to its lipoidal content, with conditions of increase of total lipoids and lecithin and relative diminution of cholesterol.

Of the more recent theories implicating lipoids in the causation of malignant tumors, that of Sokoloff is essentially similar to the views of Reinke and Meyer, in that he regarded the primary change in the inception of malignancy as one of alteration of cellular membranes and of intracellular lipoids, with a change of lipoidal equilibrium that makes cancer not so much a disease of the cell as one of intracellular relationships. Crile regards as the fundamental feature of living tissues the accumulation of free energy on the dielectric lipoidal films that separate the nucleus, the cytoplasm and the intracellular spherules. According to his theory, cancer cells must have a large electrical storage capacity, with a conductivity different from that of normal cells. He stated that an actual test showed this high dielectric capacity in cancerous tissues—a finding that is in accord with that of Fricke and Morse—and a correspondingly high conductivity, these accounting for the abnormal growth energy of cancer. The experiments of Waterman, while they indicate a disturbance of electrical relationships within the cancer cell, do not bear out this conception of Crile. Waterman found that in attempts to measure the electrical resistance of living tissues by means of the Wheatstone bridge, using an alternating current and a telephone receiver, there can be found no definite minimal tonal point, owing to polarization at cell limits. This can be offset by suitable condensers, and by means of these it is possible to measure the potential of this polarization. If the relationship of polarization to resistance is expressed by the fraction P/W, in tumors this is very small, often in fact 0. If W is expressed in terms of its reciprocal, conductivity, according to Waterman's experiments P/W, which now becomes PC, is very small, while according to Crile's hypothesis, with both capacity and conductivity high, it should be large. Waterman explained his results as due to heightened permeability, which permits both anions and cations to migrate freely and equally—an alteration which, like Crile, he ascribes to lipoidal disturbance. In connection with the action of calcium salts on cancerous tissues, Waterman had found that the effect of these is to raise the ratio P/W. As a possible explanation of the altered lipoidal metabolism that appears to be rather definitely established in malignant tumors, Corran and

Lewis found that in cancer serum there is less of lipolytic co-ferment than in that of normal individuals. Rondoni, working on the theory that restriction of lipolysis would restrain proliferation in tumors, attempted to achieve this by the injection into tumor-bearing rabbits of lipoidal mixtures, but got at best limited restriction of growth of tar cancers.

The discovery announced by Freund and Kaminer in 1910, that the serum of noncancerous persons had the property of destroying cancer cells—a property that was lacking in that of sufferers from cancer—was confirmed by Neuberg in the following year and was ascribed by him to the presence in cancerous serum of antiferments, produced in response to the discharge into the circulation of ferments originating in the cancer; this was in accordance with the finding of Freund and Kaminer that cancerous serum could inhibit the lytic action of normal serum. Kraus, Graff and Ranzi found that a similar effect was manifested by placental serum, as well as by that of gravid women up to the tenth month after the inception of pregnancy, after which the lytic property gradually reappeared. While Freund and Kaminer were inclined to view the phenomenon as one associated with predisposition to cancer, these later workers, like Neuberg, considered it as due to metabolic alteration incident to the development of rapidly growing tissues. Subsequent finding by the original discoverers that the change was not constant (they reported its occurrence in 88 per cent of 113 cases of cancer), and that it disappeared for a time after the extirpation of the cancer, was in accord with this view, although in a later report Freund and Kaminer reversed their finding of reappearance of lysis after removal of the tumor. In subsequent publications, Freund and Kaminer established the fact that the process was scarcely to be regarded as one of ferment activity, but was due, they believed, to the presence of certain fatty acids, from the effects of which the malignant cells in the cancerous subject were protected by an abnormal nucleoglobulin. The presence of this they still regarded as one of the essential elements in predisposition to cancer. The dicarboxylic acids, which appeared to be the potent lytic agent, they considered as a product of normal intestinal digestion, and the neutralizing agent as the result of production there of unsaturated fatty acids, which, joined to nucleoglobulin and carbohydrates, formed complexes which had that effect. To chronic irritation they assigned the rôle of local exhaustion of the lytic acids, as well as the possible local accumulation of the neutralizing nucleoglobulins. The parallelism between cancer and embryonic tissues in this connection which had been noted by Kraus and his co-workers was confirmed by Rosenthal, who found marked similarity in the behavior of both carcinomatous and fetal serums and tissues, as did Kraus and Ishiwara. Hirschfeld made the significant observation, as concerns the relations of

the phenomenon to cancerous predisposition, that in animals with implanted tumors the action of the serum was the same as that of individuals with spontaneous tumors. On the other hand, as corroborating its significance in predisposition there are the findings of Nather and Orator, and of Peracchia, that the lytic property normally tends to disappear in persons at about the forty-fifth year of age, although it may be absent in younger persons as well; also the fact reported by Freund and Kaminer, that the lytic property for cancer cells of the skin may be destroyed by the application of irritants of cancerogenic type, as tobacco juice or pyridine, and the observation of Waterman and de Kromme that in the induction of tar cancers there is a final complete abolition of the serum lysis, preceded by a lessening of it during the precancerous stages. In addition to being a property of normal serum, the lytic power is shown by tissue extracts, as found by Kaminer and Morgenstern, and Waterman, de Kromme and Lemmens. The former observers found that it was possessed to an unusual degree by thymic extracts, although it was absent in the thymuses of cancerous individuals. Waterman and de Kromme found it in especial abundance in reticuloendothelial tissues; they also found that its activity was limited to a narrow range of hydrogen ion concentration, and that it was sensitive to irradiation.

Attempts at identification of the agent yielded to Freund and Kaminer, as previously stated, the conception of it as a dicarboxylic acid, similar in many respects to maleic acid, but of considerably higher molecular weight. Goldzieher and Peterfi found that suberic and sebacic acids have a cytolytic action very similar to that of the agent in question. Waterman, de Kromme and Lemmens, on the other hand, were unable to identify it as an aliphatic acid, and regarded it as a combination of nucleoproteid and lipoidal material. Like Freund and Kaminer, they could find no relationships between its behavior and that of known ferments. The test has received some investigation from the point of view of possible utilization as a general and much needed diagnostic reaction. Although Koritschoner and Morgenstern found that it could be demonstrated by refractometric methods, it does not appear to be sufficiently constant to be reliable in the diagnosis of cancer. Coca found that the cytolytic action of normal serum is so dependent on uncontrollable factors as to make the test unreliable; Frankenthal found that the reaction is neither constant nor specific; Leitch found the test of no practical diagnostic value, and Herly was unable to find that normal serum applied to the Flexner-Jobling rat carcinoma had any deleterious effect on the transmissibility of that tumor. In a more recent publication, Waterman, de Kromme and Lemmens asserted their belief in the importance of the reaction in relation to predisposition to cancer; as concerns the character of the lytic agent, they considered it as akin to other

immune bodies such as occur to some extent spontaneously, and would place it among the lipoproteins.

CARBOHYDRATE METABOLISM

The earlier literature on cancer contains little reference to its relations to carbohydrate metabolism, although Rogers suggested in 1903 that this phase of metabolism might be connected with the cause of malignant growth, basing his hypothesis largely on the presence in tumors of glycogen, which had been reported by Brault in 1894 as occurring in them more or less proportionately to their rates of growth, and on the fact that Buxton had found in them quantities of amylase. The evidence for increased glycogen content was questioned in 1907 and 1908 by Haaland, who with mouse tumors could find no relationship between its presence and the rate of growth. Chisholm in 1910 and 1911 described a study of the respiratory exchange of mice in which tumors had been implanted; ordinarily there was little difference between these and normal mice, but after a carbohydrate meal there was a rise of respiratory quotient and in the excretion of carbon dioxide, which was slightly greater in the mice with tumors. A rather significant finding was reported by Cramer and Lochhead in 1913, to the effect that in tumor-bearing rats there was a more rapid exhaustion of hepatic glycogen, without, however, any indication of increased oxidative activity. They inferred that the carbohydrate was used for the synthesis of new protoplasmic material. Rhodenburg, Bernhard and Krehbiel announced in 1919 that they had found an unusual persistence of blood sugar in cancerous patients after the feeding of dextrose, with a characteristic tolerance curve which they believed might have a diagnostic value—a finding which has since been confirmed by Foerster and Foerster, who, however, also found this change in other forms of debility as well. DeNiord and Schreiner in the same year could find no characteristic changes in blood sugar nor in diastatic activity of the blood. In a later report, Rohdenburg and his colleagues stated that persons with cancer showed in larger proportion moderate reductions of blood sugar than did normal persons—a finding that was particularly likely to be the case with those showing hepatic and gastro-intestinal cancers. Lewis in 1922 observed that in cultures of fibroblasts the presence of dextrose was necessary if rapid degeneration was to be avoided, although excess of the sugar led to undue acidification of the medium.

In 1923 Warburg, in company with Minami, announced what may be surmised to be the most fundamental discovery in connection with the metabolism of cancer—the possession by carcinomatous tissue of the property of splitting dextrose into lactic acid to a degree at least 70 times that of normal tissues. An even greater effect was obtained if

respiratory activity was inhibited, as by narcotics or hydrocyanic acid, and the energy released in this process was comparable with that resulting from respiration. In later papers, Warburg, with Negelein, Posner and Wind, supplemented the original statement with the results of more detailed study. Even in an atmosphere of oxygen, the energy of malignant tumors is derived in part by glycolysis. A number of normal body tissues were found to share partially in the property, and embryonic tissues particularly, although with these there is almost complete cessation of permanent glycolysis in aerobic conditions. The only normal tissue that manifests the property of aerobic glycolysis is, rather curiously in view of its limited capacity for regeneration, that of the retina. In the process 1 molecule of dextrose yields 2 of lactic acid; with an ample supply of the former, this activity may be maintained indefinitely by the tumor, and after it has been stopped, as by freezing with liquid air, further growth appears to be impossible, as tested by transplantability. While malignant tumor tissues would appear to get the greater part of their available energy from this glycolysis, a certain amount of accompanying respiratory activity would appear to be essential, as Warburg found that the capacity of survival in strict anaerobiosis is limited to about twenty-four hours if sufficient dextrose is available; when both types of metabolism are interfered with, survival is for less than four hours. An especially interesting relation is shown by benign tumors, with which it was found that while they show the property of anaerobic glycolysis to a degree comparable with that of cancer and embryonic tissues, their power of permanent glycolytic cleavage in the presence of oxygen is relatively slight. A certain amount of aerobic glycosis may occur in normal tissues in conditions of partial asphyxiation; but here it is accompanied, as is not the case with malignant tissues, by progressive degeneration and eventual death. That a certain amount of residual respiratory metabolism is necessary for the indefinite survival of tumor tissues has also been found by Wright.

A characteristic feature of this metabolic phenomenon is the discharge of lactic acid from the cells concerned, in which respect there is a marked difference from the more usual processes of lactic acid metabolism. Rostock in 1921 had found that the injection of lactic acid into mice in which tumors had been implanted caused a marked increase in the virulence of the tumors, and Tomita observed that in the developing chick embryo there was a rapid increase of this acid until the fifth day of development, after which it diminished, apparently from exhaustion of available dextrose. Bierich in 1923 noted that the infiltration of surrounding tissues by cancerous cells was preceded there by a peculiar swelling of the connective tissue fibrils, which he was able to simulate artificially by the application of lactic acid solutions. He regarded these changes as more or less essential precursors of the

infiltration by tumor cells. There are as well a number of more direct evidences of the production of lactic acid by tumors in vivo. Glaessner, after the injection of dextrose into rats in which carcinoma or chondroma had been implanted, observed the urinary elimination of lactic acid, although this did not occur in animals in which sarcoma had been engrafted. He later reported similarly positive findings in carcinomatous persons and in mice in which cancers had been implanted. Cori and Cori, who found that the free-sugar content of malignant mouse or rat tumors was less than that of the normal tissues of those animals, were able to raise it by an induced hyperglycemia, and in these circumstances observed an increase of lactic acid to 3 or 4 times its normal level. Also, by a method of diffusion from actually circulating blood, they were able to find increased lactic acid both in sarcomatous fowls and cancerous persons. Lasnitzki found that a sarcoma of the rat induced by the injection of tumefacient bacteria produced lactic acid to an average of 7.4 per cent of the dry tissue weight, per hour, while in the normal liver and spleen of this animal the production ranged from 0.3 to 3.5 per cent. Direct estimations of the lactic acid of the general blood in cases of cancer have failed to yield significant results. Valentin found that this was somewhat increased, as did Schumacher, but only in cases of pancreatic or hepatic involvement. Mendel, Engel and Goldschneider studied the relations of blood lactic acid in the normal individual, and found that this tends in the resting state to assume a minimal value, constant for the individual, which is temporarily increased by muscular activity. In cancer, Mendel and Baruch found no evidence of increase, even after the administration of large amounts of dextrose; but as the infusion of lactic acid itself into the circulation caused no perceptible increase, they were forced to conclude that it was destroyed with some readiness at a site as yet unknown, possibly the liver. Büttner, too, could find no definite increase of blood lactic acid in early cases of cancer, although in advanced cases it accumulated apparently when the liver became the site of metastasis. Wind and von Ottingen found similar evidence of the destruction of lactic acid in the fetus. Fahrig likewise found that in most of the cases of cancer studied by him increase of lactic acid in the blood was associated with circumstances of impairment of liver function. He pointed out that even with an observed increase, its accumulation would not necessarily be due solely to the peculiar metabolism of cancer, as other factors—deficiency of oxygen, muscular disease and hepatic impairment, all conditions that might occur secondarily in cancer—could also be concerned. More direct tests have shown, in the hands of Bierich, that the local lactic acid content increases rapidly after removal of the tumor from the body—a

phenomonon explicable by greater anaerobiosis and restriction of diffusion. Stahl and Warburg showed, in the case of a carcinoma of the human bladder, that the amount of glycolysis as measured by manometric methods was 9.9 per cent of the weight of the tumor, per hour, whereas measured by lactic acid production it was 10 per cent per hour. By means of determinations of lactic acid tolerance in rats treated by injection of sodium d-lactate, Cori and Cori estimated the rate of the production of lactic acid in transplanted tumors, and achieved results almost identical with some of those reported by Warburg as obtained by direct measurements on excised tissues—690 mg. per hour per hundred grams of tumor, as compared with Warburg's rate of 700 mg. per hour. The equivalence of dextrose utilization and lactic acid production as reported by Warburg has been confirmed further by Baker, Dickens and Gallimore. A limited amount of study of tissue cultures in this connection has given results which indicate that the same metabolic change may be detected there; Demuth and Meier found in such cultures that considerably more lactic acid is produced by rat sarcoma and mouse carcinoma than by fowl and rat fibroblasts and chicken iris epithelium.

That there are peculiar relations between malignant growth and general carbohydrate metabolism has been indicated from other sources. In 1923 Braunstein called attention to the fact that with the development of cancer in diabetic patients there is not infrequently a disappearance of the glycosuria, and that in spite of the destruction of pancreatic tissue incident to carcinoma of that organ, there is seldom the appearance of diabetes in this condition. Braunstein offered confirmation of Warburg's work, inasmuch as in relation with the phenomena just noted, he studied the glycolytic activity of cancerous tissue and found that it could in most cases destroy from 30 to 40 per cent of available dextrose in from 12 to 24 hours. In a later and somewhat more detailed report, he announced that incident to the rapid growth of cancer in diabetic persons there is a lowering of blood sugar, and that the presence of urinary sugar in such cases might at times recur after excision of the cancer. Other work on the relations of cancer to general carbohydrate metabolism, also with reference to diabetes, is that of Roffo and Correa, who found that cancer in diabetic persons tends to run an accelerated course.

Although Warburg's findings have been questioned by Bauer and Nyiri on the basis of work done by them on the formation of lactic acid in tumors, there is a rather considerable body of more or less directly corroborative evidence. Okamoto found that while sarcomas and carcinomas differ much in their ability to survive in conditions of anaerobiosis, the power of survival of the latter can be considerably increased if there is a supply of dextrose available. Mahnert, and

Murphy and Hawkins in general confirmed the work of Warburg, although they did not get as clearcut differentiation into the several metabolic types of glycolysis; rat placenta, for instance, showed the anomalous behavior of malignant tissues—a finding that had been confirmed by Loeser, with opposite findings by Fujita—while with the spontaneous tumors of mice the glycolysis was mostly similar to that of benign tumors. Rona and Deutsch, and Rosenthal and Lasnitzki, also confirmed Warburg's results, and reported that glycolysis by malignant tumors was greater in anaerobic than in aerobic circumstances, indicating that the peculiar behavior of malignant tumors constitutes, on the whole, a quantitative peculiarity—an opinion that has been shared by Fahrig, Pentemalli and Crabtree, the latter with reference to regenerating tissues. Louros reported results somewhat at variance with those of Rona and Deutsch, in that the same values were obtained with anaerobic as with aerobic glycolysis in 15 cases of cancer, while in 9 there was an actual reduction of glycolysis in anaerobic conditions. Baker found that leukocytes are capable of aerobic glycolysis, in which respect his results are at variance with those of Warburg and a number of other workers. Blanchetiere found, by a technic different from that of Warburg, that tumor tissues utilize dextrose, but in greater quantity than that necessary for the amount of lactic acid produced, from which he inferred other phases of dextrose metabolism as well as the one emphasized by Warburg, whose most striking findings, in Blanchetiere's opinion, were in part due to associated sepsis. Krontowski and Bronstein found that rapidly growing tissue cultures show energetic use of dextrose, and that this is particularly marked with those of carcinomatous origin. Rondoni found it possible to accelerate the development of the precancerous epithelial changes of tar painting in rabbits by the injection of dextrose. Crabtree, who, as stated, regarded the aerobic glycolysis of tumor tissue as less a qualitative than a quantitative change, found that the relations between respiration and glycolysis show complicated relations to the sites of implantation of malignant tumors, with, however, the single constant factor of a high rate of aerobic glycolysis. The possible objection to the significance of Warburg's work on the ground that it was done almost wholly with excised and so abnormally situated tissues has been met by the work of Tadenuma, Hotta and Homma, who determined the sugar content of tumor-afferent and tumor-efferent blood, with the finding of a decided reduction in the latter, especially in circumstances of induced hyperglycemia. Cori and Cori found that the induction of hyperglycemia in tumor-bearing animals results in the elevation of the lactic acid of the blood to 3 or 4 times its normal value.

Efforts to connect the peculiar carbohydrate metabolism of tumors with known elements in that metabolism have not yielded anything

in the way of definite success. As concerns the relations of insulin to tumor metabolism, Silberstein, Freud and Revesz found that animals with tar or implanted cancers are more susceptible to insulin intoxication, but this applies only to the earlier stages of tumor development, and with large tumors the reverse relation may be observed. Rather significantly, however, the injections of insulin were found to hinder the growth of implanted tumors, and if given in very large amounts, to greatly reduce the incidence of successful "takes." Piccaluga likewise observed retardation of engrafted tumors by the injection of insulin, and Münzer and Rupp got some, though rather indefinite, evidence of restraint of growth of tar cancers by its injection, an observation which Boivin was unable to confirm, while Barral reported the directly contrary result of reestablishment of growth of apparently quiescent implanted sarcoma in the rat after the injection of small doses. That its administration would presumably result in diversion of dextrose from the tumor to other sites would appear to be indicated by Borghi, who found a marked reduction of the glycogen of tumors after its injection. As to the presence of insulin in tumors, Cori found it in traces in extracts of both benign and malignant tumors; Cramer, Dickens and Dodds, on the other hand, could not detect it in malignant tumors, and suggested that its absence might be a factor in their peculiar metabolism. Roffo and Correa were at variance with both groups of workers just cited, in that they reported the presence in considerable amounts of an insulin-like substance in a spindle cell sarcoma of the rat, although in a later report they stated that in the Jensen rat sarcoma its presence could be demonstrated only by the use of enormous amounts of tumor material. In both benign and malignant tumors of men they found it in relatively large amounts.

With regard to glutathione, Hammett would ascribe the peculiar metabolism of tumors entirely to their possession of sulphydryl compounds capable of alternate combination with and liberation of hydrogen. He based his hypothesis largely on phenomena observed in growing tissues—the precipitation of lead in them, apparently as a sulphur compound, in regions of greatest growth, as observed in root tips; the inhibition of growth by lead, and the fact that he and Reimann found evidence leading them to believe that thiodextrose, a dextrose compound containing the S H radical, operates to stimulate wound healing, as acid extracts of root tips stimulated root growth. The action of trauma in stimulating repair he would ascribe to the liberation of such sulphydryl compounds, and the cancerogenic action of tar, he believed, is due to its possession of them. Baker found that by the addition of glutathione and hemoglobin to a mixture of casein digest, glycocoll and nucleic acid, a medium is provided in which

sarcomatous fibroblasts grow for a time as well as in embryonic extracts, and his explanation is that the glutathione and hemoglobin function in vitro by the regulation of oxidation-reduction reactions within the cell as well as in the medium. Findings similar to those of Hammett with regard to growing plant tissues have been reported by von Euler and Johanson, who prepared from yeasts substances which greatly accelerated fermentation by those organisms. Hammett's results and conclusions, however, are adversely criticized by Morgulis and Green, who were unable to find any effect of various sulphydryl compounds on regeneration in the polychaete worm, Podarke obscura. There is no decisive evidence that compounds of the type of glutathione are greatly increased in cancerous growths. Bierich and Kalle found an increase of cysteine to appear progressively in both normal and tumor tissues after excision, and in organs of animals with large tumors they found no considerable reduction of glutathione. In general there would appear to be approximately the same amount of glutathione in malignant tumors as in normal tissues. While Yaoi and Nakahara found that the Rous tumor was very poor in respect to this, LeCloux, Viviani and Firket found in transferable mouse tumors more of it than in the corresponding normal tissues, but even so less than is found in the liver or in embryonic tissues. In their opinion, a high glutathione content does not connote malignancy, but simply an excess of cellular metabolism. Kennaway and Hieger found that the content of glutathione varied with the amount of cellular material, and was independent of the normal or neoplastic character of the tissue. Bierich and Kalle, in addition to the indirect evidence cited here, found the sulphydryl contents of normal and malignant tissues approximately alike. Holmes, on the other hand, found that malignant tumors of the rat and of man contain abnormally small amounts of glutathione, and in rat tumors she found that added glutathione was very slightly reduced, while Voegtlin and Thompson found in transferable mammalian tumors a high glutathione content, comparable with that of the liver, one of the organs normally richest in that substance. As regards the relations between reduced and oxidized glutathione, Voegtlin and Thompson found a higher than normal ratio of GS-SG to GSH in tumors, while Bierich and Kalle found that while as a rule GS-SG is greater than GSH, this relationship is reversed in cancer, as it is in liver, muscle and lens substance.

In connection with the relation of glutathione to cancerous predisposition, Voegtlin and Thompson found that the total content of this in the body diminishes with age, a fact that was observed also by Murray during the embryonic life stages of the chick. In view of the fact, however, that Hammett considered the relation of malignant growth to the sulphydryl compounds as one of increased sensitivity to them in

the tissues concerned, rather than as a response to quantitative increase, neither this nor the failure to demonstrate in tumors excessive amounts of glutathione are necessarily decisive arguments against his hypothesis. A theory apparently almost the opposite of that of Hammett is that of Grumme, who would seek the cause of cancer in sulphur impoverishment, on the grounds of elimination of tar and arsenic as sulphur compounds, high elimination of sulphur in cancer cases as reported by Salomon and Saxl, etc.

Other views of the nature of the unusual behavior of tumors with respect to carbohydrate were suggested by Waterman, who in 1924 reported that with tumor extracts he was able to enhance the glycolytic activity of normal tissues; apparently, from the results of Dische and Laszlo, this effect may be manifested within the tumor host, as they found that the hepatic tissue of carcinomatous mice showed increase of glycolytic power, although the kidney, the organ with which Waterman obtained his results, did not. According to Aldus, Chiari and Laszlo, the glycolytic agent of mouse carcinoma is carried in the filtrate, and is absent in the liver of those animals, and Landegger and Pirker found in the serum of cancerous patients, as well as in that of pregnant women and in that of some normal persons, a fermentation-accelerating substance which they regarded as essential for the development of cancer. Kraut and Bumm, on the other hand, while finding no increase in the glycolytic power of tumor filtrates, found, like Waterman, that these have the power of greatly enhancing glycolysis by normal tissues, having an effect in this respect of co-ferment rather than of ferment activity. Embryonic extracts were found by them to stand midway between normal tissues and malignant tumors in this regard, and the glycolytic activity of tissues appeared te be proportional to their possession of this co-ferment. Rosenthal found that a similar co-fermentative action may be developed by normal tissues when kept in vitro in aerobic conditions, as was indicated by their subsequently increased rate of aerobic glycolysis—a phenomenon that tumors failed to show with respect to dextrose, although it occurred in them with relation to fructose. Neuberg, Kobel and Laser were able to obtain from rat sarcoma by extraction with alcohol and ether or with acetone an active glycolase, but the co-ferment was destroyed during the process, so that while the ferment acted on hexose-phosphate, the methylglyoxal formed was not further changed, indicating the importance of the co-ferment in the later stages of carbohydrate cleavage only. As Lasnitzki pointed out, the aerobic glycolysis of tumor cells may be simulated by dying cells, but with the difference that in the one circumstance reproduction precedes death. He pictured the alteration of metabolism as a gradual one, of a similar character to what has been observed in partially asphyxiated embryonic chicks, in which monstrous

development follows from arrest of developmental processes to a greater degree than those of growth, but with the outstanding difference that in the case of malignant growth the alteration is irreversible. Warburg's explanation of the change that he discovered is on the whole the simplest; he pictured malignant tumor cells as formed of descendants of exceptional cells with unusually retained powers of the glycolysis normally possessed during the embryonic state, in circumstances in which they are forced to rely more and more entirely on glycolysis as a source of energy, and in which their more normal fellows perish. Still another view of the nature of tumor glycolysis was advanced by Bierich, who expressed the opinion that the accumulation of lactic acid in and about malignant tumors is essentially due to inability of the tumor tissues to resynthesize the lactic acid into dextrose in a manner similar to what occurs in more normal sites of lactic acid production.

PHYSICAL CHEMISTRY

In addition to the electrical phenomena reported by Waterman, changes in surface tension, permeability, etc., have been given a considerable amount of study in relation to cancer. That alterations in the character of surface contacts of cells would produce changes in their interrelations and modes of growth was shown by Loeb in 1902, in a series of experiments in which he transplanted epithelial cells into a colloidal medium (agar or blood plasma) within the animal. He observed changes suggestive of carcinomatous development, particularly in the way of abnormal cellular arrangements—incidentally in these experiments taking the first step toward the independent culturing of tissues. In 1914 Lambert observed that in tissue cultures the dilution of the plasma with isotonic salt solution had the effect of promoting more extensive cellular migration, without, however, showing any effect on the rate of cellular multiplication. In regard to intracellular fluid content, Cramer found that in both normal and cancerous tissues this was increased in circumstances of growth and was more or less proportional to the rate of multiplication; the increase he did not ascribe to increased osmotic tension, but to differences in imbibition. Larson, Cantwell and Hartzell showed that the growth of bacteria in nutrient broth is greatly affected by the surface tension of the medium, even to the extent that with some anaerobes growth could occur in aerobic conditions in mediums of reduced surface tension, though too great a reduction acted adversely. A somewhat similar effect of freer growth was observed with cultured normal fibroblasts by Hogue, in that these showed an enhancement of growth rate in hypotonic salt solution, along with a shorter period of cell survival.

A number of workers have reported a lowering of surface tension of the blood or serum of cancerous subjects—Roffo and Kopaczewski, Svehla, Bauer, Solowiew and Lederer, the latter two finding a similar though less pronounced variation during pregnancy as well. Svehla would associate the reduction of surface tension with a lowering of blood calcium content, as he believed he could establish direct relationships between the two. Sauer, on the other hand, was unable to find any essential differences between the surface tensions of serums of cancerous and noncancerous gynecologic patients. As regards cancer tissues, Kagan reported that their extracts show as a rule reduction of surface tension, as did Solowiew. Bauer approached the problem indirectly, with a study of the effect of reduced surface tensions on dividing cells, using for these fertilized eggs of Ascaris, and reported that with them the diminution of surface tension effects a marked promotion of cellular reproduction. Bauer, indeed, believed that the local lowering of surface tension is the fundamental factor in the causation of cancer. His theory is based on the following postulates: Cancerogenic action must operate on the surroundings of the cells concerned; it must tend toward their isolation; it must promote cellular division; it must be possessed in common by all known cancerogenic agents, and it must be universally present in cancerous growth—demands all of which he asserted are met by reduction of surface tension. He extended his theory to embrace even the metastatic colonization of malignant tumors, as he found that the expressed juice of spleen has a relatively high surface tension, that of muscle tissue an intermediate value, and those of lymph gland, liver and kidney relatively low values. Krontowski, Bereschanski and Majeroski investigated alterations of surface tension in tissue cultures, and found that in the medium there was no appreciable reduction during tissue growth, with the exception of a mouse carcinoma in which this occurred to a slight degree. However, in circumstances of aseptic cellular disintegration a marked reduction took place, and they suggested that this may be the mechanism that acts in regeneration, to say nothing of malignant growth. Kagan found that reduction of surface tension by the treatment of cancer tissue with tributyrin greatly enhanced its growth after implantation, and Katzenstein and Knake reported that substances that lower surface tension have the effect, in tissue cultures, of stimulation of epithelial, and suppression of connective tissue, growth; certain tissues, however, as liver, behaved exceptionally. Einaudi's finding that neoplastic cells in general have a greater volume than that of the parent cells, and a particularly greater nucleocytoplasmic ratio, would apparently fit in with the conception of lowered surface tension as a major factor.

To the extent that the presence of calcium salts operates to restrain growth, a possible explanation is offered by the finding by Magath

that after-treatment with isotonic calcium chloride solution the imbibitory power of tumor cells is greatly reduced. Such results are strikingly analogous to those obtained with collodion membranes by Brinkman and Szent-György. They found that treatment of these with alkaline salts of fatty acids rendered them permeable to dissolved hemoglobin, an effect that was irreversibly abolished by calcium chloride. Mendelieff reported that normal cells, after disturbance in a number of ways —by the application of tar or trypoflavine or by exposure to the x-rays —show a series of changes in permeability—an initial increase, followed by diminution even below normal, and a final return to the normal or a hypernormal condition—while cancer cells do not show these variations and maintain a constantly high permeability. Magath reported similar findings. Lange and Henning, in a study of cellular elimination of phosphoric acid, found that the output of this was greatly enhanced in conditions of anoxybiosis, and that in tumors there was, on the basis of such elimination as evidence, a regular increase of cellular permeability. Magath called attention to the fact that the effects of the x-rays on cellular permeability are paralleled by the known imbibition phenomena of cancer and by those of electrometric polarization as measured by Waterman, and that all indicate the association of cancer with alterations of cellular permeability. The similarities between the "forcing" of plants and cancerous growth, to which attention has been called by Boreschi, would appear to admit of explanation along the lines of alterations in permeability, but of impairment rather than augmentation of this. In both there is inhibition or limitation of respiration, along with an accumulation of acid products. Niethammer, working with a new forcing agent, mercuro-phenol-mercury, found that this, like the excitants of animal cell proliferation, does not penetrate into the cells, but inhibits respiration by surface action; this in turn is associated with the intracellular accumulation of acetaldehyde, hydrocyanic acid, reducing sugars and amino-acids, with a cumulative effect of elevation of total acidity—all changes that he associated with impairment rather than facilitation of intercellular exchange.

Recently Kostoff suggested that the cause of cancer is to be sought in physicochemical changes in the cytoplasm which have the effect of modifying cellular behavior without affecting reproductive function. As an illustration, he adduced the production of tumors in hybrid tobacco plants by the injection of extracts of one of the parent plants, in cases in which the two parents showed reciprocal power of protein precipitation.

GENERAL SUMMARY

It is altogether obvious that in spite of the tremendous volume of work that has been done in connection with cancer and its inception,

there is as yet no theory of its origin which is adequate to meet all the known facts, and which is supported by sufficient direct evidence to render it satisfactorily probable. This is not to admit, however, that the work already accomplished has been fruitless. To a certain extent, its value lies in its negative character; as a result of it, one may conclude with some certainty that there are some things which, from the etiologic standpoint, cancer cannot be.

First, it may be considered as definitely established that cancer is not infectious in the sense that its origin is due to the attack of a specific parasite. It is of course well proved that certain infections may cause malignant growth, but these are so diverse in character that to explain their effects on the common ground of parasitism would necessitate viewing them as carriers of a specific virus, rather than as causative agents in themselves. It is quite true that this view has been advocated, most notably by Borrel, and the peculiar experience of Blumenthal with certain of his tumefacient organisms would lend it support. But the production by A. Fischer of a malignant tumor with fibroblastic cells cultured in vitro, by purely chemical agencies—supported as this experiment is by the somewhat similar, but from the present point of view, less decisive, experiments principally of Carrel—would appear to exclude definitely this hypothesis from serious consideration. Even the Rous filtrable virus, which most nearly meets the conception of an independent causative agent, would appear from this experiment of Fischer to be of metabolic rather than parasitic character, even if the probability of this had not been recognized from the extreme specificity of the several viruses of this type. If more evidence is needed of the essentially nonparasitic origin of cancer, it is afforded by the work done on production of cancer by the various irritants. True these, too, might be explained as acting through a local reduction of resistance to a conjectured parasite. But the certainty with which some of them induce cancer in suited subjects; the practically complete nonsusceptibility of other animals even though closely akin phylogenetically to those that are susceptible, and as subject as they to malignant tumors in different circumstances; the character of some of the irritants themselves, particularly those involving radiant energy, along with the fact that in suitable animals, as the rat, the effect of the latter agencies is ordinarily on deep rather than on superficial tissues—these, in connection with the experiment of Fischer already cited, would lead to the almost certain conclusion that the effect of all these agencies is a direct one, and that the known cancer-inducing parasites are simply special types of cancerogenic irritants.

With reference to the older theories of the induction of cancer, the more recent experimental work has thrown little light on the validity of the so-called Cohnheim's hypothesis, and has even to a limited extent

given it some support. Experimentally, malignant tumors have been produced on the whole more readily with embryonic than with adult tissues. To the extent that this theory may be linked with modern information of cancer metabolism, as has been done by Warburg, it is impossible to say that it may not meet the facts adequately. The evidence against this theory is largely indirect. In view of the localized action at the point of application of most of the cancerogenic irritants, to explain their effect on these grounds would involve a conception of almost ubiquitous embryonic displacement, or more properly, perhaps, of persistence of cells of embryonic potentialities. Just as altogether too little is known of metabolic phenomena in individual cells to be able to say with any certainty that none of them differs in metabolic potentialities from its fellows to the extent demanded by Warburg's hypothesis, so, too, present ignorance does not permit one to assume this as an established fact. What is essentially a modern adaptation of Cohnheim's hypothesis, then, at present is and seems likely to remain a possible but not directly provable speculation.

In this connection, a theory recently advanced by Simons may be of some interest, even though it is quite largely of speculative character. He regards cancer as resulting from the stimulation of persistent embryonic cells in response to bodily need for a growth principle elaborated in them. It is somewhat reminiscent of one advanced by Ishihara, who believed that all the phenomena of cancer may be explained by a deficiency in a hormone found in umbilical jelly and placental tissue; but it is supported by a modicum of experimental evidence, since Simons found that in his experience wound healing was accelerated by insertion of embryonic tissues.

As to the theories that derive the cancer cell from the results of nuclear fusion, with, as offspring, cells of abnormal potentialities, there does not appear to be any foundation for them in the facts revealed by experimental, as distinct from morphologic, investigation. Since it has been possible to produce malignant tumors in vitro in circumstances that would appear to exclude absolutely the possibility of such fusion, these theories may be dismissed as vain speculations. The same cannot be said of von Hansemann's theory of anaplasia, which nothing in the field of experimental study especially contradicts; however, as has been emphasized repeatedly, the theory is one of mechanism rather than of cause.

Regarding theories such as that of Ribbert, which would ascribe malignant growth to the abnormal relations incident to cellular displacement, nothing has been found in experimental study to support them, and here the fact that unusual agencies are necessary to evoke cancerous growth in misplaced cells, as in tissue culture or within the

organism, or even in implanted embryonic cells, would seem to relegate cellular displacement to a place of relatively little importance.

It would appear to be necessary to explain cancer as the result of metabolic anomaly, by which the cells involved achieve the property of unlimited and unrestricted growth as the result of perversion of their normal functional and nutritive relations. This view has been suggested frequently in the many theoretical speculations on the origin of cancer. It was advanced as long ago as 1886 by Steven, and since, in somewhat variant forms, by numerous others. In part it has been represented as a diversion of the normal expenditures of energy of the cell from its miscellaneous functions to the one demand of reproduction. Morphologic studies of most adenocarcinomas would give ground for such a view, but in circumstances in which one can evaluate more accurately the persistence of functional activities on the part of cancer cells, as in those of the endocrine glands, there would appear to be a frequent retention of special function in spite of the excessive expenditure of energy in cellular reproduction. It is much more probable that the energy change is not one of diversion, but rather of additional source, as suggested by the work of Warburg. To this enhancement of cellular reproductive activity a large number of factors appear to contribute, either solely or in summation—inherent growth energy, as of embryonic tissues, in which, as pointed out by Warburg and his associates, there is a rather considerable resemblance to cancer cells in their capacity to obtain energy by glycolysis; achieved growth energy, as from chronic regenerative changes, in which the same exceptional source of energy occurs; hereditary predisposition; secretory or endocrine imbalance. To these must be added an element or elements of as yet unknown character, since in the matter of chronic regenerative changes alone, those induced by certain, "cancerogenic" agencies, are effective with some regularity, while others result in cancer only rarely, and then apparently in the presence of other concomitant factors.

Concerning the mechanism by which this metabolic aberration is brought about, there are much speculation and little knowledge. It has been ascribed to the action of necrohormones—growth-stimulating substances liberated from dead and dying cells. It has been attributed to hormonal imbalance, by Shirlaw, Fichera, Walz, Haberland, and in part by Willy Meyer, and others. While, as has been shown by Loeb, hormonal disturbances do contribute to the occurrence of cancers in laboratory animals, they would appear to be only one of the numerous factors concerned in their causation, and there is little definite evidence in support of the view that they are of primary importance. The same observation is true of the attempts to explain cancer solely on the grounds of vitamin abnormality. Burrows and Jorstad would assign

the regulation of cellular reproduction to the interaction of a growth-restraining principle, which they regard as more or less identical with vitamin A, and a stimulant agency regarded as homologous with vitamin B. To the extent that the experiments of Saiki and Fujimaki and Erdmann and Haagen in the induction of cancers by imbalance of these vitamins indicate their importance in the causation of cancer, the comparatively low incidence of positive results would seem to denote that their effect, like that of so many other factors, is only a contributory one; nor is there any indication in the results of Drummond that there is any appreciable quantity of vitamin B either in tumors or in actively growing normal tissues.

It would seem that there is more knowledge of the nature of the aberration than of its mechanism. From the work of Warburg it is rather definitely established that abnormally excessive carbohydrate cleavage is the source of the extra supply of energy necessary for the excessive growth. But along with this there appear to be associated other factors—lowered surface tension, increased cellular permeability and a series of phenomena apparently related to this, namely abnormal lipoidal relations, electrical reactions and response to calcium salts, as well as added intracellular water content. Recent work would indicate an abnormal, or abnormally excessive, co-ferment as the primary factor in this special cleavage of dextrose, and to what extent this may be dependent on these other changes, and which of the several associated alterations is to be regarded as the primary one, with the others ensuing in sequence to it—of all this one is in complete ignorance.[1]

The problem is not necessarily unsolvable. In view of the developments of less than the last decade, it does not seem vain to hope that further experimental work will eventually correlate into a logical and connected whole the several peculiarities that characterize the behavior of the cancer cell, and furnish a clear and well established conception of the nature and origin of malignant growth.[2]

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1. In a rather recent summary of his views, Auler expressed the belief that cancer may result from a number of different mechanisms. Among these he included: embryonization of cells; cellular isolation, which may result from anomaly, trauma, chemical irritation, senility, chronic inflammation, hormonal abnormality, etc.; factors of individual predisposition, with, in his opinion, contributary to this an element of intestinal abnormality that results in intermediate products of polysaccharid digestion which in turn interfere with the cleavage of the monosaccharids; other elements, such as increased alkalinity of the blood, a relative increase of potassium salts and an altered content of fibrinogen and globulin in the blood, and anaphylactic disturbances affecting the nerve and hormonal supply to the part involved.

2. To save space, the bibliography has been omitted. The author will be glad to furnish any references to the literature cited in his review on the etiology of cancer that the reader might desire. It is contemplated that the review will appear in monograph form, and in this case, the list of references will be included.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Frederick E. Becker, assistant professor of pathology in the school of medicine of the University of Colorado, has died at the age of 43.

Roscoe R. Hyde has been appointed professor of immunology in the School of Hygiene and Public Health of the Johns Hopkins University to succeed the late Carroll G. Bull.

J. Florey has been appointed professor of pathology, and J. W. Edington professor of bacteriology, in the University of Sheffield, England.

Phillips Prize.—The American College of Physicians has awarded the first Phillips Prize of $1,500 to Oswald T. Avery of the Rockefeller Institute for Medical Research for his work on the pneumococcus.

Provision for Surgical Research.—One million dollars has been bequeathed to the Boston City Hospital by the will of the late Charles H. Tyler for the establishment and maintenance of a laboratory for surgical research to be known as the George E. Sears Laboratory.

Society News.—The next annual meeting of the American Association of Pathologists and Bacteriologists will be held in Philadelphia on April 28 and 29, 1932. One half-day will be given to the discussion of tuberculosis and one half-day to a joint meeting with the American Association of Immunologists. The meeting will be in conjunction with that of the Federation of American Societies for Experimental Biology.

Abstracts from Current Literature

Pathologic Anatomy

THE CEREBRAL LESIONS IN PURULENT MENINGITIS. FREDERIC WERTHAM, Arch. Neurol. & Psychiat. **26**:549, 1931.

In studying the cerebral changes in twenty-four cases of purulent meningitis, Wertham centered his attention on pale areas (clearings) in the cortex and white substance. In these areas many ganglion cells either dropped out or appeared shrunken and homogeneous; their nuclei were pyknotic, while other cells showed severe changes. The glia in the pale areas exhibited either regressive or progressive changes, depending on the location of the lesion. In the cortex, the pale areas (the clearings) were small, affecting one or more layers. The glia seemed to have been increased and appeared as clusters. The nerve fibers were tortuous and swollen, and at the junction of the white matter and cortex they were demyelinated. The olives appeared especially vulnerable, whole portions of which dropped out, and many ganglion cells disappeared. Of interest also were the ventricular changes, in the form of a wall-like formation of the ependymal layer, cufflike infiltrations of the blood vessels and subependymal proliferation of the glia. The blood vessels showed changes in both the meninges and the parenchyma — perivascular infiltrations and some splitting of the elastic membrane. The vascular changes as well as toxic substances that may affect the blood vessels and the parenchyma alike are held by Wertham responsible for the pale areas that he believes may account for the cerebral manifestations in patients who recover from purulent meningitis.

GEORGE B. HASSIN.

CONTINUOUS OBSERVATIONS ON LIVING GRANULATION TISSUE. J. TANNENBERG, Verhandl. d. deutsch. path. Gesellsch. **26**:145, 1931.

By a modification of the method of Clark-Sanderson, a celluloid chamber was inserted into the rabbit's ear, and therein the development of vascular granulation tissue was studied over a period of from six to eight weeks. The new method permits the use of high magnifications, even of oil immersion and the making of motion pictures. A photomicrographic film is presented, which shows all stages from the new formation of blood vessels to dense connective tissue. By application of moderate heat and light, the emigration of leukocytes from the blood vessels was demonstrated.

C. ALEXANDER HELLWIG.

THE BLOOD MONOCYTES IN INFLAMMATORY EXUDATE AND IN GRANULATION TISSUE. W. BUENGELER and B. FISCHER-WASELS, Verhandl. d. deutsch. path. Gesellsch. **26**:148, 1931.

India ink and other colloidal solutions were injected into rabbits for a period of from two to four weeks. Then, by intravenous injection of protein, monocytosis was produced. Venous blood was withdrawn from different organs: aural vein, femoral vein, inferior vena cava, renal, portal and hepatic veins, splenic vein and right and left ventricles. Analysis of the blood showed the greatest number of monocytes present in the liver and spleen. The hepatic vein contained from forty to fifty times more monocytes than the vein of the ear. There was the greatest difference in the number of monocytes between the right and the left ventricle, a fact indicating that many monocytes originating from the spleen and liver are retained in the capillaries of the lungs. Many of the monocytes were stained with india ink and were therefore regarded as reticulo-endothelial cells.

In rabbits which were prepared in the same way as in the first experiment, granulation tissue was produced. Many monocytes were found that were stained with india ink. These cells behaved, furthermore, exactly like true blood monocytes when supravital stain was applied (janus green and neutral red). The authors conclude from their experiments that monocytes of the blood and in granulation tissue are identical; i. e., they are reticulo-endothelial cells.

C. ALEXANDER HELLWIG.

THE PATHOLOGIC SIGNIFICANCE OF RESIDUE STRUCTURES IN MICROSCOPIC SECTIONS. W. GERLACH, Verhandl. d. deutsch. path. Gesellsch. **26**:163, 1931.

Systematic examinations were made of residue structures in microscopic sections of heart, lung, liver, spleen, thyroid gland, muscle, aorta and testis in 80 cases in which autopsy was performed. The results were not satisfactory. There are cases poor, as well as cases rich, in residues. The amount of residue increases with the age of the patient, but there are also young patients with large amounts of residues. The findings are inconstant in cases of tuberculosis and carcinoma. The chemical analysis is incomplete except for calcium, iron and perhaps potassium. The author therefore prefers the spectrographic method devised by Gerlach-Muenchen by which a photographic spectrogram is obtained from fresh material evaporated in the high frequency spark. In a case of silicosis, sections of the lung showed the presence of silicon, aluminum, calcium, magnesium, iron, copper and silver. In a minute specimen of gingival mucosa in a case of lead poisoning, lead was plainly detected by this method. In gallstones, copper was demonstrated. The distribution of copper and manganese in cirrhotic liver was shown to be very irregular.

C. ALEXANDER HELLWIG.

EXPERIMENTAL STUDIES ON THE EXCRETION OF URIC ACID. A. SCHULTZ, Verhandl. d. deutsch. path. Gesellsch. **26**:174, 1931.

Saturated colloidal solutions of sodium and lithium urate were injected intravenously into rabbits. After ten and thirty minutes, respectively, the left and right kidneys were removed and fixed in trinitrophenol alcohol. Paraffin sections were stained with methylene blue, by which the uric acid deposits stain a brilliant green. Within a few minutes after injection, abundant deposits of uric acid were found in the following portions of the kidney: (1) the capsules of Bowman; (2) the epithelial cells of the uriniferous tubules, especially the straight tubules, and the ascending parts of Henle's loops; (3) the lumen of the whole loop of Henle and the excretory tubes. It is the first time that the excretion of uric acid by the glomeruli has been demonstrated.

C. ALEXANDER HELLWIG.

THE COPPER CONTENT OF THE CIRRHOTIC LIVER. E. VON ZALKA, Verhandl. d. deutsch. path. Gesellsch. **26**:180, 1931.

Nineteen livers without evidence of cirrhosis contained from 0 to 8 mg. of copper per thousand grams. The copper content in thirty-four cirrhotic livers was determined. The values varied between 1.87 and 95.25 mg. (average 31.59 mg.) per thousand grams. In fourteen cirrhotic livers, or 41 per cent, the values were normal, namely, below 10 mg. In five of thirteen livers presenting Laënnec's atrophic cirrhosis, the amount of copper was increased, while four of seven livers showing Laënnec's hypertrophic cirrhosis had a higher copper content. A liver showing tuberculous cirrhosis showed a content of 24.4 mg.; another showing syphilitic nodular cirrhosis, 52.08 mg.

The facts that the copper content of the liver is not increased in every case of cirrhosis of the liver, and that, on the other hand, a large amount of copper may be found also in biliary, tuberculous and syphilitic cirrhosis, are not in favor of the theory that copper is an important factor in the etiology of cirrhosis. It is more likely that the high copper content is due to the retention of the metal

in the diseased organ. It is assumed that there is some relationship between the copper content of the liver and the function of the reticulo-endothelial cells in the diseased liver.

C. ALEXANDER HELLWIG.

IDIOPATHIC CYSTIC MEDIAL NECROSIS OF THE AORTA. J. ERDHEIM, Virchows Arch. f. path. Anat. **276**:187, 1930.

Erdheim believes that many of the reported cases of so-called spontaneous rupture of the aorta may have been due to the lesion described by him. The principal changes, which are at first limited sharply to the media, are necrosis and mucoid degeneration. The process may lead to the formation of relatively large mucoid cysts. Regenerative changes become associated with the degenerative ones, but there is no cellular exudation or infiltration. The ascending portion of the aorta is chiefly involved, but necrotic mucoid patches have been encountered throughout the entire course of the aorta. All the elements of the media, muscle, elastic tissue and connective tissue take part in the reparative process, the muscle being most active. Regenerated areas of the media are easily recognized by the preponderance of muscle and by the abnormal shape and course of the muscle fibrils. The regenerated tissue is not a physiologically adequate one and readiiy undergoes degeneration in its turn. In the regenerated areas, elastic fibrils are relatively few and small and stain faintly. The connective tissue is relatively somewhat diminished in amount. The etiology of the process is doubtful. Endogenous and exogenous intoxications, that with nicotine being included among the latter, have been considered causative factors. Experimental work suggests a possible relation to avitaminosis. Hyperadrenalinemia seems to be an important factor.

W. SAPHIR.

REORGANIZATION IN THE WALLS OF CALCIFIED ARTERIES. G. B. GRUBER, Virchows Arch. f. path. Anat. **275**:54, 1930.

Gruber presents a histologic study of five examples of ossification of calcified arteries. He limits the term organization, as used in his title, to the replacement of dead tissue by living tissue that participates in the general metabolism of the body, and leaves out of question any physiologic and functional regeneration of the vessel. The specific matters that engage his attention are the question whether fracture of calcified arterial plaques occurs during life, the mechanism of removal and absorption of calcium from the arteriosclerotic plaques, the process of bone formation in such areas, the formation of the cartilage occasionally seen, the reaction of calcium resorption to ossification and the question whether the ossification is to be considered a new stage in the arteriosclerotic process. That fracture of calcified plaques occurs during life is proved by the presence between the ends of the fragments of proliferating mesenchymal tissue, which has been likened to the callus of fractured bone. Proliferating mesenchymal granulation tissue, which is formed between calcium fragments, and which may invade unfractured calcified material from the walls of the vasa vasorum, has the property of removing calcium by absorption and by phagocytosis. A syncytial foreign body giant cell tissue may be formed. The mesenchymal tissue may differentiate in several different directions. It may form fibrillated connective tissue, adipose tissue or marrow. From it may be developed osteoblasts that form bone. Gruber believes that bone formation in calcified arteries occurs through osteoblasts and rarely, if ever, by direct ossification of fibrous tissue. Cartilage likewise is formed by differentiation of the mesenchymal tissue. The cartilage may reveal evidences of both resorption and transformation into bone. For the resorption of calcium and for the fragmentation and removal of dead calcified material, proliferating mesenchymal tissue is necessary. Whether this tissue differentiates into bone appears to depend on local physical and chemical factors. Whether the bone formation that may occur in calcified arteries is to be considered an essential stage in the arteriosclerotic process depends on the latitude with which arteriosclerosis is defined. The bone formation is in no way characteristic of arteriosclerosis. O. T. SCHULTZ.

Experimental Pathology and Pathologic Physiology

LOCAL TISSUE REACTIONS DUE TO VARIATIONS IN ION CONCENTRATION. K. AKAMATSU, Beitr. z. path. Anat. u. z. allg. Path. **85**:348, 1930.

Attempts to determine tissue changes at the interface between living tissue and solutions of different ion concentration meet with difficulty because of rapid changes in ion concentration due to the quick diffusion of the solution into the tissues. To overcome this difficulty Akamatsu devised the ingenious method of preparing small gelatin blocks, each containing a central air bubble. The blocks were hardened in formaldehyde solution to prevent their solution in the animal body, and were washed free from formaldehyde in running water. The solution the action of which was to be studied was injected into the air bubble contained within the gelatin block, and the latter was placed in the peritoneal cavity of a guinea-pig under aseptic precautions. The present report relates to the results obtained with 5 per cent and 2 per cent hydrochloric acid and with 5 per cent and 2 per cent potassium hydroxide. In preliminary experiments, the prepared blocks were kept in a moist chamber at body temperature, and the reaction tested at intervals with indicators. Blocks prepared with 2 per cent hydrochloric acid lost the acid reaction at the surface in fourteen days; with 5 per cent hydrochloric acid in seventeen days. Blocks prepared with 2 per cent potassium hydroxide retained an alkaline reaction for nineteen days, and those with 5 per cent potassium hydroxide for twenty days. Animals into the peritoneal cavities of which the prepared blocks had been introduced were killed at intervals varying from six hours to twenty-one days after introduction. A generalized peritoneal reaction was not set up. Such changes as occurred were limited to the tissues that were in contact with the surface of the block. The acid solutions caused a more rapid and more marked adhesion of the adjacent omentum or mesentery to the surface of the gelatin block than did the alkaline solutions; the latter frequently caused no adhesion whatever. Acid caused a greater and more diffuse emigration of leukocytes to the interface between tissue and gelatin block than did alkali. Leukocytes wandered into the central cavity of the acid blocks; this phenomenon was not observed in the alkaline blocks. The leukocytes in contact with the surface of an acid block had markedly lobulated and deeply stained nuclei that appeared degenerated. The leukocytes in contact with the alkaline blocks had larger and less deeply stained nuclei, with few lobulations.

O. T. SCHULTZ.

THE INFLUENCE OF COAL AND STONE DUST ON TISSUE CULTURES. A. LAUCHE, Verhandl. d. deutsch. path. Gesellsch. **26**:107, 1931.

The method of tissue cultures was utilized for the study of pneumonoconiosis. Lauche devised a new apparatus that allows one to sprinkle dust on tissue cultures evenly and in the finest particles. By covering part of the culture with small glass bells, the simultaneous dusting of one culture with two different kinds of dust was accomplished. Comparative studies with various substances showed that common coal dust is more avidly engulfed by phagocytes than pure animal coal, and much better than iron dust. The phagocytosis of stone dust is most sluggish.

When coal and stone dust were sprinkled on tissue cultures at the same time, the former increased strikingly the intensity of phagocytosis of the stone particles. This phenomenon is explained by substances adsorbed by the coal which cause a greater motility of the phagocytes.

C. ALEXANDER HELLWIG.

TISSUE CULTURE OF SO-CALLED ALVEOLAR EPITHELIUM FROM TUBERCULOUS LUNGS. F. HENKE and M. SILBERBERG, Verhandl. d. deutsch. path. Gesellsch. **26**:114, 1931.

Silver stain does not permit a definite distinction between epithelial cells (alveolar epithelium) and mesenchymal cells of the septums, because the latter cells also take the silver stain. The authors studied, therefore, the behavior

of these cells in tissue cultures. They infected rabbits with bovine tubercle bacilli intravenously and cultivated the lung tissue from two to four days after infection. From observations on the living tissue it was apparent that the so-called large exudate cells in tuberculosis of the lungs behave exactly like mesenchymal cells. First they develop into different forms of histiocytes and finally into ripe fibrocytes. Giant cells can be seen in twenty-four hour cultures, where they form by confluence of exudate cells. The epithelioid cells of the tubercle are derived from the mesenchymal "alveolar phagocytes." The authors accept this term, which was recommended by Seemann in place of alveolar epithelium, because they conclude from their experiments that the alveoli of the lungs are not lined by a continuous layer of true epithelial cells.

C. ALEXANDER HELLWIG.

THE FORMATION OF BILIRUBIN IN TISSUE CULTURES. E. VON BALOGH, S. SUEMEGI and M. CSABA, Verhandl. d. deutsch. path. Gesellsch. **26**:118, 1931.

To 730 tissue cultures of different organs a solution of hemoglobin was added, and from four to five days later, the indirect van den Bergh test was performed on the cultures. The formation of bilirubin was noticed from three to four days after the hemoglobin had been added to the cultures taken from the spleen, brain, spinal cord and pericardium of 18 day old chicken embryos and from the iris, the medullary portion of the adrenal glands and the spleen of guinea-pigs. The van den Bergh reaction was negative in cultures made from the heart muscle, cartilage of the ribs, wall of the veins and skeletal muscle. The authors conclude from their studies that bilirubin forms extrahepatically in several organs, most abundantly in the spleen, and that its formation depends on certain cells (reticuloendothelial cells) present in these organs. Hematoidin and bilirubin are closely related.

C. ALEXANDER HELLWIG.

THE GROWTH RATE OF TISSUE CULTURES IN DIFFERENT PREPARATIONS OF EMBRYONIC EXTRACT. G. BORGER and R. ZENKER, Verhandl. d. deutsch. path. Gesellsch. **26**:124, 1931.

Chick embryo extract, carefully dried in the vacuum and dissolved in water, has the same growth-stimulating effect on tissue cultures as fresh extract. After keeping the dried product for five months, the authors found it as effective as fresh extract, when used in fibroblast cultures.

The growth-stimulating substances of embryonic extract are apparently proteolytic ferments. The same processes, such as heating to 60 C. or shaking, which lower the growth-stimulating properties of embryonic extract, were shown to decrease the amount of proteolytic ferments in the extract.

C. ALEXANDER HELLWIG.

THE HYPERPLASIA OF THE UTERINE MUSCLE DURING PREGNANCY. B. FISCHER-WASELS and W. BUENGELER, Verhandl. d. deutsch. path. Gesellsch. **26**:129, 1931.

The anatomist Stieve concluded from histologic examinations of the pregnant human uterus not only that the growth of the uterine muscle during pregnancy is due to a hyperplasia of the preexistent muscle fibers, but that new unstriated muscle cells form from fibroblasts, histiocytes and adventitial cells, and even from lymphocytes of the blood. The authors do not believe that the histologic method will ever be able to solve this problem. They examined the uterus of the mouse after the injection of pituitary hormone from the urine of pregnant women. One hundred hours after the injection of the hormone the uterine muscle showed the same hypertrophy as at the end of a natural pregnancy of twenty-one days. By this method the whole process of muscle growth can therefore be studied in short intervals, and it was demonstrated that the uterine muscle hypertrophies exclusively

from preexistent muscle fibers, by very active mitotic division of unstriated muscle cells. Also by using vital stains, Fischer-Wasels and Buengeler found that no other mesenchymal or blood cells participate in the growth of the pregnant uterus.

C. ALEXANDER HELLWIG.

EXPERIMENTAL ARTHRITIS DEFORMANS. F. KLUGE, Verhandl. d. deutsch. path. Gesellsch. **26**:216, 1931.

In the rabbit, true arthritis deformans will develop from anaphylactic arthritis. The disease process was studied in many x-ray pictures during the course of the experiment, over a period of three years, and on histologic sections obtained in different stages of the disease.

In rabbits or other animals, when repeated injections of heterologous blood serum, for instance, from the horse or dog, were made into one knee joint, anaphylactic arthritis, which resembled polyarthritic rheumatism, developed. After about six months, without further injection of foreign serum, a chronic hyperplastic synovitis, periarthritis and osteochondritis developed in the knee joint into which the injection was made. Still later, about one year after the beginning of the experiment, no inflammatory changes were noticeable, but there were destructive processes that resembled, anatomically and in the x-ray picture, typical arthritis deformans: degeneration and even complete loss of the superficial cartilage, vascularization of the cartilage, marginal exostoses, osteoporosis and osteosclerosis.

C. ALEXANDER HELLWIG.

Microbiology and Parasitology

SPONTANEOUS AND EXPERIMENTAL INFECTION OF PIGEONS WITH B. AERTRYCKE. J. R. CASH and C. A. DOAN, Am. J. Path. **7**:373, 1931.

The apparently spontaneous development of a fatal disease in undernourished pigeons is reported which is characterized by anemia, marked myeloid hyperplasia of the bone marrow, striking increase of the myeloid elements of the blood and extensive infiltration of the liver and kidneys with myeloid tissue. In addition to these myeloid changes, large, nodular, often necrotic masses of mononuclear phagocytic cells are frequently found scattered throughout the liver, spleen, kidneys and bone marrow. A small, gram-negative bacillius, regularly recovered in pure culture from the blood, liver, kidney, spleen and bone marrow in these cases, has been identified as B. aertrycke. In sections, the bacteria are found to be present in the foci of mononuclear cells, but do not occur within the collection of myelocytes. Disease has been produced experimentally in normal pigeons by the intraperitoneal injection of liver emulsion made from naturally infected birds, by intraperitoneal injection of B. aertrycke derived from the same source and also by oral administration of single large doses of broth cultures of this organism. Bacteria-free filtrates of broth cultures of B. aertrycke have had no demonstrable effect on normal pigeons when injected or administered orally in single large doses. Attention is called to the frequency with which pathologic changes occur in the tissues of apparently normal birds.

AUTHORS' SUMMARY.

CEREBRAL CHANGES IN A FATAL CASE OF CHICKENPOX. H. M. ZIMMERMAN and HERMAN YANNET, Arch. Neurol. & Psychiat. **26**:322, 1931.

The patient, aged 13 months, died in convulsions on the fourth day of chickenpox. There were diffuse changes in the ganglion cells (vacuolation of cell bodies) of the cortex, basal ganglions, pons and medulla, lipoid accumulation in the adventitial spaces of the blood vessels in the white matter of the parietal and occipital lobes, with areas of demyelination around the blood vessels, and presence of fat granule bodies in the subarachnoid space. Inflammatory phenomena such as plasma cells or lymphocytes were lacking.

GEORGE B. HASSIN.

CATAPHORESIS EXPERIMENTS WITH PROTEIN-FREE SUSPENSIONS OF A BACTERIOPHAGE AND FOWL-POX VIRUS. I. J. KLIGLER, L. OLITZKI and M. ASCHNER, Brit. J. Exper. Path. **12**:178, 1931.

Cataphoresis experiments with purified suspensions of E. coli bacteriophage indicate that this agent is negatively charged in neutral and slightly alkaline reactions but amphoteric in acid or strong alkaline solutions. In broth suspensions this agent migrated to the positive pole at all reactions. The fowl-pox virus is positively charged on the acid side, is amphoteric in neutral solutions, and carries a negative charge in alkaline solutions. The bacteriophage and fowl-pox virus in purified suspensions are highly sensitive to acid reactions. C. E. CLIFTON.

THE FILTERABILITY OF VACCINIA VIRUS. A. B. GREEN and G. H. EAGLES, Brit. J. Exper. Path. **12**:202, 1931.

The authors claim that vaccinia virus passes readily through Berkefeld V filters and to some extent through Berkefeld N and English Berkefeld filters. The p_H of the virus suspension (6.4-8.4) does not influence the results, and lining the filters with acidified egg white or the use of hormone broth as a dilutent is not necessary. These filtrates can be kept in the cold for a week or more without marked deterioration in activity. C. E. CLIFTON.

ULTRAFILTRATION STUDIES ON THE VIRUS OF POLIOMYELITIS. C. E. CLIFTON, E. W. SCHULTZ and L. P. GEBHARDT, J. Bact. **22**:7, 1931.

A simple procedure is presented for the preparation of aceticcollodion membranes for ultrafiltration studies, and the principles of ultrafiltration are discussed. A method is described for the removal of the lipoids and part of the associated proteins from suspensions of the virus of poliomyelitis. Ultrafiltration studies on this purified suspension indicate that the magnitude of this virus lies below 50 millimicrons in diameter. AUTHORS' SUMMARY.

ETIOLOGY OF TRACHOMA. U. LUMBROSO, Arch. Inst. Pasteur de Tunis **20**:137, 1931.

A complete presentation of studies of twenty-eight cases of trachoma is given. The methods of Noguchi, adapted to the cultivation of Bact. granulosis, were used, as well as various other procedures designed to recover unknown or previously described organisms. Detailed protocols show the frequent isolation of streptococci and staphylococci, and in no instance the isolation of Bact. granulosis. However, in eighteen or twenty-eight cases small bacilli, culturally somewhat different from Noguchi's organism, were recovered. The ordinary pathogenicity of these organisms was nil, and data were not yet at hand concerning their action on the conjunctiva. These organisms were grouped into types A, B and C, based on morphologic and biochemical studies. M. S. MARSHALL.

BACT. GRANULOSIS AND TRACHOMA. CHARLES NICOLLE and UGO LUMBROSO, Arch. Inst. Pasteur de Tunis **20**:239, 1931.

Bact. granulosis (Noguchi and Olitski strains) have shown themselves, in our hands, to have lost all pathogenicity, both intravenously injected and in the conjunctiva. By the latter route the Olitski organism induced a very light and very transitory follicular reaction; the Noguchi organism produced nothing. In no case was trachoma produced. To these results of rigorous experiment only one objection may be presented, the date of isolation of the cultures used, the aging of cultures altering the virulence of all organisms. AUTHORS' CONCLUSIONS.

SYMPTOMLESS OCCURRENCE OF TUBERCLE BACILLI IN TONSILS WITH RECURRENT ARTHRITIS AND RETROBULBAR NEURITIS. K. AMERSBACK, A. LÖWENSTEIN and E. LÖWENSTEIN, München. med. Wchnschr. **78**:1078, 1931.

Tubercle bacilli were cultured from the tonsils of five patients in a group of fourteen with polyarticular rheumatism. Cultures of the blood were negative. Clinically these patients had no symptoms of tuberculosis. Similar studies in five patients with retrobulbar neuritis, of whom one had the classic symptoms of multiple sclerosis, demonstrated tubercle bacilli in the blood of the patient with multiple sclerosis, and in a second patient tubercle bacilli both in the blood and in the tonsils. The organism in the latter patient was of the avian variety. These results are published preliminary to a more extensive study.

AUTHORS' CONCLUSIONS.

TUBERCLE BACILLEMIA IN DISEASES OF THE CENTRAL NERVOUS SYSTEM. E. LÖWENSTEIN, München. med. Wchnschr. **78**:1080, 1931.

Tubercle bacilli were not cultivated from the blood of 3,000 healthy persons. They were cultured from the blood of patients with chorea, retrobulbar neuritis, multiple sclerosis and schizophrenia without demonstrable visceral tuberculosis.

AUTHOR'S SUMMARY.

RETICULO-ENDOTHELIAL VITAL STORAGE IN EXPERIMENTAL TUBERCULOSIS. W. PAGEL and J. E. GARCIA-FRIAS, Virchows Arch. f. path. Anat. **275**:479, 1930.

The authors report the results following the injection of india ink into guinea-pigs recently infected and superinfected with tuberculosis, the experiments being undertaken primarily for the purpose of studying the responses of the allergic animal. In experimental tuberculosis of the liver, the giant and endothelioid cells of the tubercles stored large quantities of the carbon particles. Storage by these cells was more marked than by the Kupffer cells of the normal and regenerated portions of the liver. The tuberculous process experimentally induced was not altered in any way by repeated injections of india ink over long periods of time or by extirpation of the spleen. Storage was not influenced by the local or generalized allergic reaction to tuberculin. Carbon storage had no influence on the local tuberculin reaction of allergic animals. In such animals the injection of tuberculin caused a more active exudative inflammatory response than did the injection of india ink alone or the injection of the ink and tuberculin in normal animals. The presence of stored carbon appeared to inhibit somewhat the formation of tuberculous granulation tissue following superinfection or a local tuberculin reaction. When india ink and tubercle bacilli were injected at the same time, removal of the stored carbon was more rapid than when the same procedure was carried out in the already infected animal. In the former, abscess formation occurs and the carbon is removed from the area of injection in a relatively short time, whereas in the allergic animal the particles are held for a long time by the cells at the point of injection. Experimental tuberculosis afforded very definite protection against serum anaphylactic shock. The degree of protection depended somewhat on the degree of generalization of the tuberculous process, on the extent to which the lungs and spleen were involved and on the amount of necrosis and caseation. The protection was greater after the generalization of the infection and moderate tubercle formation following subcutaneous injection than after the more rapid generalization and more widespread tubercle formation following intravenous injection of tubercle bacilli. The protection against serum anaphylactic shock could be passively transferred by the injection of organ pulp of tuberculous animals. The serum of such animals had a less marked protective action, and tuberculin, emulsion of tubercle bacilli and brei of normal animals had little or no protective action. The protection against active serum anaphylaxis is ascribed to an altered reaction to toxic substances brought about by the generalization of the

infection and the formation of tubercles. Passive protection is ascribed to protein split products. Exposure of tuberculous animals to roentgen rays, the generalized tuberculin reaction or the injection of iodine compounds, lipoid extracts or drugs on the sympathetic system caused no decrease in the protection afforded by tuberculous infection to serum anaphylaxis. The local tuberculin reaction of the allergic animal sensitized to serum was increased by the simultaneous injection of tuberculin and serum. Serum sensitization did not render normal animals more susceptible to tuberculin.

O. T. Schultz.

The Pore Size and Sieve Action of Bacterial Filters. H. Bechhold, Ztschr. f. Hyg. u. Infektionskr. **112**:413, 1931.

Evidence is presented to show that the diameter of the pores in a bacterial filter must be from eight to fifteen times as large as the longest diameter of a microorganism that will pass through the filter. Protective colloids did not increase the filterability of the agents studied. Nonspore formers will grow through filter pores of double their greatest diameter, while spore formers and branching forms will grow through even smaller pores.

C. E. Clifton.

Immunology

Nature of the Viricidal Antibody in Antipoliomyelitis Serum. E. W. Schultz, L. P. Gebhardt and L. T. Bullock, J. Immunol. **21**:171, 1931.

Though viricidal antibodies were easily demonstrated in the serums from human beings and monkeys convalescent from poliomyelitis, no evidence could be elicited to indicate that these antibodies are related to antibacterial antibodies. The presence of complement-fixing and precipitating antibodies specific for the virus could not be demonstrated even in serums from hyperimmunized convalescent monkeys. On the other hand, evidence was obtained that strongly suggests that the antibodies parallel antitoxins in their behavior. Like neutral toxin-antitoxin, neutral serum-virus mixtures may be rendered infective again by dilution with physiologic solution of sodium chloride. Moreover, while an undiluted serum of sufficient titer may render a given quantity of virus material immediately innocuous, when such a serum is diluted a certain minimum of time is required to accomplish the same result.

Authors' Summary.

Properties of a Bouillon Filtrate of the Gonococcus. L. T. Clark, N. S. Ferry and A. H. Steele, J. Immunol. **21**:233, 1931.

The gonococcus produces an extracellular toxin, when grown in suitable liquid medium that promotes rapid development. This toxin is contained in the sterile bouillon filtrate from young cultures in sufficient concentration to give positive skin reactions in dilutions of from 1:1,000 to 1:1,500. Injection of the toxin into animals stimulates the formation of an antitoxin that neutralizes the toxin in vitro as well as in vivo. Control autolysates and mechanically produced control intracellular extracts give negative skin reactions in low dilutions. An "antivirus" meeting the specifications of Besredka was not produced in liquid cultures of the gonococcus allowed to incubate for from six to seven days.

Authors' Summary.

A Specific Antigenic Carbohydrate of Type I Pneumococcus. A. Wadsworth and R. Brown, J. Immunol. **21**:245, 1931.

From type I pneumococci a specific substance, apparently carbohydrate, was isolated. Its properties and antigenic activity are described as follows: Precipitation occurred with type I antipneumococcus serum in a dilution of 1:6,000,000. With type I antipneumococcus serum from which the antibodies against the specific

carbohydrate of Heidelberger and Avery had been adsorbed, precipitation occurred in a dilution of 1:600,000. Complement was fixed in the presence of type I antipneumococcus rabbits serum. Tests for protein were negative, and those for carbohydrate, positive. Mice immunized with the substance were protected against type I pneumococcus infection. It caused fatal anaphylactic shock in guinea-pigs passively sensitized with type I antipneumococcus rabbit serum; also, in guinea-pigs passively sensitized with type I antipneumococcus rabbit serum from which the antibodies against the specific carbohydrate of Heidelberger and Avery had been absorbed.

AUTHORS' SUMMARY.

PYROGENIC REACTION TO ANTIPNEUMOCOCCUS SERUMS. L. D. FELTON and GLADYS KAUFFMANN, J. Infect. Dis. **49**:337, 1931.

Chill-producing lots of antipneumococcus immune serum contain material precipitable at a p_H value of from 4.8 to 5.2, whereas under these conditions good preparations contain little. Chill-producing lots have a slightly higher phosphorus and perhaps a higher lipoid content than good ones; the differences are so slight, however, as to indicate that the residuum of these agents in the final preparations may not be responsible for pyrogenic action. Unsatisfactory preparations contain more ammonia and nonprotein nitrogen than do those that are suitable for intravenous injection.

AUTHORS' SUMMARY.

ARTIFICIAL FEVER AND EXPERIMENTAL POLIOMYELITIS. C. W. JUNGEBLUT and N. KOPELOFF, J. Infect. Dis. **49**:348, 1931.

Noninfectious fever of considerable intensity applied during the period of incubation and maintained over a short period of time is not capable of altering the course of experimental poliomyelitis in the monkey to any significant extent. The same holds true for infectious fever produced by inoculation with Trypanosoma equiperdum, when the two diseases are induced simultaneously. With poliomyelitis infection superimposed on existing trypanosomiasis there may be distinct prolongation of the period of incubation of the poliomyelitis infection, undoubtedly indicating some attenuation of the virus. The latter observation is based on one monkey. In poliomyelitis it would seem that the production of a sufficiently intense fever to bring about destruction of the virus in vivo is not possible within a safe zone of raised temperature.

FROM AUTHORS' SUMMARY.

AGGLUTININ ABSORPTION IN UNDULANT FEVER. E. FRANCIS, Pub. Health Rep. **46**:2416, 1931.

The chief interest in these studies centers about the serologic reactions of certain cultures of Brucella abortus (Bang). A brucella that manifests the cultural character of requiring carbon dioxide for its isolation is Br. abortus (Bang), and yet certain cultures of such an organism are shown by agglutination to give the melitensis A serologic reaction of Br. melitensis (Bruce). Any contention that agglutinin absorption is a reliable test for the differentiation of Br. abortus (Bang) from Br. melitensis (Bruce) is not supported by these studies. Abortus and melitensis cultures may be quickly, but only tentatively, separated serologically by their agglutination in one or the other of the following previously absorbed serums: (1) an abortus type serum which has been absorbed by a melitensis type culture or (2) a melitensis type serum which has been absorbed by an abortus type culture. The result of such an incomplete test is not to be taken as final evidence, but only as suggestive.

AUTHOR'S SUMMARY.

THE WEIL-FELIX REACTION IN TSUTSUGAMUSHI DISEASE. J. W. WOLFF, J. Hyg. **31**: 352, 1931.

The occurrence of the Weil-Felix reaction in a series of forty-five cases of tsutsugamushi disease (type Schüffner) is recorded. The agglutination reaction

was negative in fifteen cases, the other thirty showing a positive reaction with the "Kingsbury" strain of B. protex X. The Weil-Felix reaction is not regarded as adequate for differentiating tsutsugamushi disease from scrub typhus, since reactions of high titers (1:1,000) occur in a considerable proportion of cases of tsutsugamushi disease. In the present state of knowledge of the two diseases it is recommended that they should be separated according to the presence or the absence of a primary ulcer and bubo.

AUTHOR'S SUMMARY.

RABICIDAL ANTIBODIES IN RABBITS IMMUNIZED AGAINST RABIES. G. STUART and K. S. KRIKORIAN, J. Hyg. **31**:414, 1931.

In the immune serums of rabbits treated with killed, etherized-fixed virus, rabicidal antibodies make an earlier appearance, are present in greater degree and persist a longer time than in the immune serums of rabbits treated with equal quantities by weight of fresh-fixed virus or of killed, carbolized virus.

AUTHORS' SUMMARY.

FOOT AND MOUTH DISEASE IN VACCINE LYMPH. T. VAN HEELSBERGEN, Ann. Inst. Pasteur **46**:558, 1931.

Norwegian vaccine lymph no. 980 produced foot and mouth disease, as well as the usual reaction to the vaccine. The possibility of dual properties in a single virus was ruled out by the successful separation and preparation of pure live virus strains, each producing the symptoms known to be associated with it and providing no cross-immunity.

M. S. MARSHALL.

REINOCULATION WITH B C G IN THE GUINEA-PIG. L. BALOZET, Ann. Inst. Pasteur **46**:604, 1931.

Repeated intracardiac or intraperitoneal injections of B C G did not alter the virulence of the first inoculation or the histologic changes induced by it. Deaths were frequent following intracardiac injection, and were apparently due to a toxic substance. Repeated subcutaneous inoculations caused an abscess, which, after the third or fourth injection, developed a little more rapidly, but healed rapidly. Repeated subcutaneous injections after an intracardiac injection did not inhibit the healing of lesions due to the latter; following an intraperitoneal injection, healing seemed to be favored by subsequent subcutaneous inoculations.

FROM THE AUTHOR'S CONCLUSIONS.

ANTIGENIC FAILURE OF SPIROCHAETA PALLIDA FROM TESTICULAR CHANCRES. F. PLAUT and H. KASSOWITZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:193, 1931.

The inhibiting effect of the tissue extract is made responsible for the lack of antigenic qualities both in vivo and in vitro. Extracts of spirochetes from cultures that possess antigenic qualities lose these when extracts of various tissues are added.

I. DAVIDSOHN.

THE QUANTITATIVE DEVELOPMENT OF GROUP-SPECIFIC RECEPTORS IN THE SERUM OF THE NEW-BORN INFANT. OLUF THOMSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:199, 1931.

The serum of new-born infants contains the same group receptors that are present in the red blood cells. The titer is not lower than that of the serum of adults. In cases of heterospecific pregnancies, the serum of the fetus may combine with the iso-agglutinins of the mother, which have passed through the placenta. This explanation is offered to replace the hypothesis of Hirszfeld of the selective retention of certain iso-agglutinins by the placenta. The rather weak development

of receptors in the red blood cells of new-born infants is another protective mechanism counteracting the deleterious effect of the passage of corresponding iso-agglutinins from the mother.

I. DAVIDSOHN.

DYSENTERY ANATOXIN. M. P. ISABOLINSKI and B. P. KARPATSCHEWSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:213, 1931.

To dysentery toxin, 0.5 per cent formaldehyde was added and the mixture kept six days at 48 C. Rabbits immunized with it developed a high degree of resistance to dysentery toxin. The efficiency of the anatoxin was dependent on the strength of the toxin from which it was prepared. The serums of the rabbits so immunized had only weak protective properties.

I. DAVIDSOHN.

THE INFLUENCE OF THE SIMULATANEOUS INTRODUCTION OF VARIOUS ANTIGENS ON IMMUNITY. M. P. ISABOLINSKI, W. A. JUDENITSCH and B. P. KARPATSCHEWSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:218, 1931.

Guinea-pigs infected with tubercle bacilli and subsequently treated with diphtheria anatoxin did not become resistant to diphtheria toxin as the control animals did. Injection of tubercle bacilli after resistance for diphtheria toxin had already developed resulted in a lowering of this resistance. The injection of BCG, proteus X_{19} bacilli and its toxins, antirabic emulsion and scarlet fever vaccine did not interfere with the development of immunity against diphtheria toxin. Children treated simultaneously with scarlet fever vaccine and diphtheria anatoxin developed resistance to diphtheria toxin as estimated with the Schick test.

I. DAVIDSOHN.

THE RÔLE OF THE SKIN IN THE PRODUCTION OF ANTIBODIES AGAINST SPIROCHAETA PALLIDA. F. PLAUT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:223, 1931.

Very small doses of nonvirulent, pure cultures of Spirochaeta pallida, injected subcutaneously or applied on scarified skin, stimulated the development of specific agglutinins and lysins, but no advantage was observed when these routes were compared with the intravenous. Extracts of skin from the areas into which the antigen had been injected did not contain agglutinins, indicating a lack of participation by the skin in the production of antibodies against spirochetes from cultures. Virulent spirochetes from testicular chancres did not produce agglutinins when injected intracutaneously and did not react in vitro with serums prepared against cultural spirochetes.

I. DAVIDSOHN.

THE ACTION OF BACTERIOPHAGES ON THE AGGLUTINOGEN FOR HUMAN RED CELLS IN B. DYSENTERIAE-SHIGA. M. EISLER and A. F. HOWARD, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:473, 1931.

The antigen mentioned in the title, and the Forssman antigen, which are present in B. dysenteriae-Shiga, disappear as a result of the action of bacteriophages on cultures of the organism. The agglutinability of the bacteria by their specific immune serums, which is also lost under the same conditions, is restored by growing the bacteria in alkaline broth; the agglutinogen for human red cells, however, does not return.

I. DAVIDSOHN.

THE BIOLOGIC SPECIFICITY OF THE FEMALE GERMINAL PLASMA OF THE CHICKEN. RICHARD BREIER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:477, 1931.

Chicken egg-white is species-specific and does not produce lipoid antibodies in the rabbit. Immune serum produced in rabbits with injections of chicken egg-yolk

reacts with egg-yolks of other birds, showing this antigen to be organspecific. The egg-yolk immune serum reacts with the homologous egg-white, but not with the egg-whites of other birds; it contains lipoid antibodies and reacts with alcoholic extracts of the homologous antigen and of the egg-yolks of other birds. Also general lipoid antibodies are present, giving positive complement fixation with the various alcoholic extracts used for the Wassermann reaction. Heterophilic antibodies of the Forssman type (in the form of the proper titer of antisheep hemolysin) are also present. An immune serum produced with boiled egg-yolk reacts with the homologous antigen, with the raw egg-yolk, with its alcoholic extract, and with the egg-yolks and even with the egg-whites of numerous other animals. When used for immunization, alcoholic extracts of egg-yolk behave like haptens. Immune serum produced with alcoholic extracts of lecithin react with alcoholic extracts of various egg-yolks, but not with their watery suspensions. Immune serum produced with duck egg-yolk displays immunologic reactions like those of chicken egg-yolk serum, but does not contain heterophilic antibodies, owing to the absence of the corresponding antigen in the duck.

I. DAVIDSOHN.

THE ANTIGENS OF THE RED BLOOD CELL STROMA. KEIHO KAMADA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:522, 1931.

Chemical fractions of the red cell stroma of the horse displayed differences in antigenic qualities. Complement-fixing antibodies were produced only by the "secondary ether extract," containing phosphatides and cerebroside-like substances. Hemolysins were mainly produced by the "water extract," containing albumoses, nitrogen residue substances, cerebrosides, carbohydrates and soluble proteins. The "primary ether extracts," which contained cholesterol-like substances, were distinctly less antigenic. The extraction modifies the antigenic substances, which is also demonstrated by means of proper absorptions. For use in the complement-fixation test, unchanged red blood cells are the most efficient antigen.

I. DAVIDSOHN.

THE ANTIBODIES IN COLD-BLOODED ANIMALS. M. K. EBERT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:13, 1931.

Frogs produced specific precipitins for rabbit, sheep and rat serums and specific hemolysins and agglutinins for rabbit, sheep and human erythrocytes. The production of nonspecific precipitins could not be demonstrated.

I. DAVIDSOHN.

THE PRODUCTION OF SPECIFIC SERUM WITH PARATYPHOID BACILLI. M. KURODA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:45, 1931.

Aoki and his associates have demonstrated a specific and a nonspecific variant in paratyphoid bacilli which could, in many cases, be isolated as separate cultures. Specific immune serums were produced with the help of proper absorptions. There specificity went so far that the serum did not agglutinate strains of the homologous species which did not contain the specific variant. The disadvantage of such too far going specificity was overcome by the production of specific serums that acted equally specifically on the specific and the nonspecific variants of the homologous species, but not at all on heterologous bacteria. To bring out the nonspecific agglutinins, the number of immunizations had to be increased.

I. DAVIDSOHN.

THE CONTENT OF HISTAMINE-LIKE SUBSTANCES IN THE GUINEA-PIG IN PROTEIN SENSITIZATION. K. HOSOYA and K. WATANABE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:57, 1931.

Immediately following the injection of serum, and during the anaphylactic shock, a decrease of aforementioned alcohol-soluble substances was found in the lung, small intestine and urinary bladder; in the large intestine it was noticeable

only during the anaphylactic shock. A rise of the substance was noted some time after the sensitizing injection, reaching a maximum and then declining slowly while the sensitiveness to the protein persisted without any apparent relation to the level of the histamine-like substance. The liver and striated muscles showed no changes. The temporary nature of the rise points against the hypothesis that the anaphylactic reaction in the guinea-pig is due to the liberation of a histamine-like toxic substance in the tissues.

I. DAVIDSOHN.

THE BINDING OF HEMOLYTIC ANTIBODIES. HANS V. EULER and EDVARD BRUNIUS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:65, 1931.

A study has been made of the influence of the hydrogen ion and of the sodium chloride concentration on the absorption of antisheep hemolysin by the stroma of sheep's red blood cells. The optimum p_H value was found to be about 5.3, both in the presence and in the absence of sodium chloride. At a p_H value of from 6.5 to 6.6, only 10 per cent of the hemolysin was bound if sodium chloride was absent, while if it was present, about 85 per cent was fixed. The influence of the salt was not so marked when lipoid extracts of red blood cells were used instead of stroma, but the optimum p_H value remained the same. When the antibody was bound by the lipoids, various attempts to separate the two led to a recovery of only small quantities of the former, the presence of serum being a favorable factor for the procedure.

I. DAVIDSOHN.

THE BIOLOGY OF SPIROCHAETA PALLIDA. S. BERGEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:93, 1931.

Spirochaeta pallida was injected intraperitoneally into rabbits and mice, and the changes in morphology were noted. Similar studies were conducted in vitro by mixing the spirochetes with peritoneal exudates rich in lymphocytes and with extracts of lymph glands from an infected animal. Dark field observation and various staining methods were employed. Sections from healing human and from experimental rabbit lesions were also studied. The most important change consisted in the appearance of granules in the course of the spirochetes. Tinctorial degenerative changes were seen with the use of the Giemsa stain. In sections stained according to Levaditi, the spirochetes were less numerous within and in the neighborhood of the lymphocytic accumulations and showed distinct degeneration down to granular disintegration. In agreement with others, the author assumes that Spirochaeta pallida shows an outer lipoidal coat, while the center is a lipoid-protein mixture. The Wassermann reaction is based on the presence of two kinds of reactive substances: those against specific spirochetal lipoids, and those against the nonspecific lipoids found in tissues infested with spirochetes.

I. DAVIDSOHN.

THE EFFECT OF DIMINISHED CONCENTRATIONS OF SODIUM CHLORIDE ON COMPLEMENT FIXATION. ERWIN NETER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:136, 1931.

The concentration of sodium chloride was diminished to 0.6 per cent in the antigen-antibody-ocmplement mixture. In the sheep's red cells and antisheep hemolysin mixture, a correspondingly higher (1.8 per cent) concentration of sodium chloride solution was used to obtain the concentration of physiologic solution of sodium chloride. The effect was a distinct and uniform intensification of the fixation, when immune serum against heterophilic organ extracts, against lecithin, against serum proteins or against B. coli was used with the corresponding antigen. It was shown that the effect was not due to an anticomplementary action of lower concentrations of the sodium chloride solution. The reaction was not less marked at room temperature than in the incubator; it even seemed to be more intense.

I. DAVIDSOHN.

COMPLEMENT IN NORMAL GUINEA-PIG SERUM IN VARIOUS PERIODS OF LIFE. E. FRIEDBERGER and I. GURWITZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:164, 1931.

New-born guinea-pigs had approximately the same amount of complement in their blood as their mothers, while it was absent in fetuses delivered by cesarean section. The complement obtained from guinea-pigs of various ages and weights showed no essential differences in the titers. The old custom of using guinea-pigs of a certain age and weight for complement does not appear justified by the results of this study.

I. DAVIDSOHN.

CAN CONDITIONAL REFLEXES OF PAWLOW STIMULATE THE PRODUCTION OF IMMUNE ANTIBODIES? E. FRIEBERGER and I. GURWITZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:173, 1931.

According to Metallnikow (*Ann. Inst. Pasteur.* **46**:131, 1931; abstr., ARCH. PATH. **12**:496, 1931), conditional reflexes are able to stimulate the production of specific antibodies in animals previously immunized, as shown by a rise in the titer. The present study revealed no such effect in rabbits treated with sheep's red blood cells and with cholera vibrios. The slight stimulation shown in the titer of the antibodies was not convincing and possibly was due to individual variations.

I. DAVIDSOHN.

Tumors

THE EFFECT OF TESTICLE EXTRACT AND OF NORMAL SERUM ON A TRANSPLANTABLE EPITHELIAL TUMOR OF THE RABBIT. F. DURAN-REYNALS, J. Exper. Med. **54**:493, 1931.

Extract of rat, rabbit or bull testicle prevents or retards the growth of a rabbit tumor when a mixture of the extract and a tumor cell suspension is inoculated intradermally. Similar mixtures, made with normal rabbit serum instead of testicle extract, give rise to tumors which grow with unusual rapidity. The results are the opposite of those obtained with pathogenic bacteria or filtrable viruses which are enhanced by testicle extract and generally inhibited by normal serum.

AUTHOR'S SUMMARY.

TUMOR SUSCEPTIBILITY AND HEREDITY. C. J. LYNCH, J. Exper. Med. **54**:747, 1931.

A male mouse from a strain with a high incidence of spontaneous lung tumors was crossed with several females derived from a low tumor strain. The first generation of offspring was then backcrossed to animals of the original strains. The resulting two groups of offspring differed significantly in the incidence of spontaneous tumors of the lungs. These facts are discussed in relation to others previously discovered. It seems clear from the evidence presented that there are among mice constitutional types which differ in incidence of tumors of the lungs, and that the differences are inherited. The number of genetic factors involved has not been determined. No influence of sex was apparent. The possibility of there being genetic factors that affect tumor age will be dealt with later.

AUTHOR'S SUMMARY.

CARCINOMA OF UTERINE HORN AND INTERSTITIAL PORTION OF FALLOPIAN TUBE. WARREN C. HUNTER and WILLIAM B. HOLDEN, Surg., Gynec. & Obst. **39**:746, 1931.

The study concerns a papillary adenocarcinoma in the uterus of a woman, 30 years of age, with menorrhagia almost continuously since the onset of menses, relieved temporarily by curettage, but recurring with increasing severity. The

symptoms simulated those of incomplete abortion, but microscopic examination of the material evacuated from the uterus revealed adenocarcinoma. The uterus on removal was found to contain a papillary carcinoma situated so far laterally in one cornu that it escaped the curet and was found only by making many cuts at close intervals. Microscopic serial sections were necessary to determine whether the growth arose from tubal or endometrial epithelium. The latter proved to be the origin of the neoplasm in what was in all probability at one time a benign polyp. Pressure by surrounding muscle molded the growth, preventing centrifugal extension, except into the uterine cavity. The myometrium was not invaded.

FROM AUTHORS' SUMMARY.

CHORIOCARCINOMA OF THE INTESTINE WITHOUT A PRIMARY GENITAL FOCUS. A. LAPOINTE, A. CAIN and L. DARFEUIL, Ann. d'anat. path. **8**:425, 1931.

The interesting feature in the case is that a thorough examination of the reproductive organs failed to reveal any tumor, whereas the intestines showed multiple foci of the choriocarcinoma. The patient, a woman of 30, died of a septic peritonitis from intestinal perforation about four months following a miscarriage.

B. M. FRIED.

LYMPHANGIOMA OF THE TENDON. R. HUGUENIN and C. OBERLING, Bull. Assoc. franç. p. l'étude du cancer **20**:144, 1931.

Two cases of this disease, which occurred in a boy of 3 and in a man of 47, respectively, are well reported.

B. M. FRIED.

A CASE OF PRIMARY SARCOMA OF THE CERVIX. OHLSEN, Arch. f. Gynäk. **145**:817, 1931.

A woman, 32 years old, had leukorrhea for five months and persistent metrorrhagia and abdominal pain for six weeks. A cauliflower-like growth 4 by 4.5 by 3 cm. arose from the posterior lip of the cervix, was reddish yellow and bled easily on touching. Total hysterectomy was done. No metastases or local invasion were found. The microscopic structure was that of a large round cell and spindle cell sarcoma. Mitoses were absent. The author, as well as Robert Meyer, believed it to be a malignant myoma. The patient was well one year later.

LAWRENCE PARSONS.

THE FUCHS REACTION IN THE SERODIAGNOSIS OF CANCER. B. H. E. CADNESS and C. G. L. WOLF, Biochem. Ztschr. **238**:287, 1931.

Using the Fuchs test, the authors obtained in eighteen cases of cancer tested either a positive reaction or an immunity reaction. In sixteen nonmalignant cases the test was negative in 69 per cent; in four cases of stomach ulcer and in one case of nephritis it was positive. Previous irradiation of the patient with x-rays or radium makes the test negative for apparently several weeks afterward. The proteolytic action of cancer serum is diminished after a period of five days in cold storage. The same treatment has no effect on normal serum. Fibrin kept in the icebox for six months is unchanged. The addition of heparin to the serum or the previous washing of the fibrin in 90 per cent alcohol have no effect on the reaction. Previous boiling of the fibrin decreases the proteolysis considerably. The boiled fibrin cannot be reactivated by the addition of enterokinase and calcium chloride. Phosphate buffers of p_H 7.4 and 7.1 and borate buffers of p_H 7.1 have a lysing action on fibrin, more so at 37 C. than at room temperature. The thereby produced ninhydrin positive substances do not pass through a cellophane membrane. The amount of nitrogen present corresponds with the intensity of the ninhydrin test made with the filtrate. Previous boiling of the fibrin inhibits the action of the

buffers. Precipitation of the serum with aluminum hydroxide leaves the proteolytic ferment in the supernatant fluid. The Fuchs' ferment is therefore probably of a tryptic nature or identical with kathepsin. The ferment is precipiated with the proteins, if alcohol is used.

WILHELM C. HUEPER.

EFFECT OF SODIUM FLUORIDE ON TUMOR METABOLISM. C. SELLEI and J. JÁNY, Biochem. Ztschr. **239**:94, 1931.

Tumor fermentation is inhibited by sodium fluoride in dextrose solution, but not in lactate solution. Oxidation is also interfered with by sodium fluoride, but to a lesser degree than fermentation. The fermentation of cancer tissue is decreased, but the respiration is unchanged, at high concentrations of sodium fluoride. In dilute solutions of sodium fluoride, representing from one one-hundredth to one ten-thousandth-normal, the respiration is increased, while the glycolysis is decreased. The tumor metabolism has then an aerobic character. Also, the two heat quotients of cancer tissue are similar to those present in normal tissue.

WILLIAM C. HUEPER.

ACUTE LEUKEMIA WITH A FIVE MONTHS REMISSION. HENRY JACKSON, JR., FREDERIC PARKER, JR., G. P. ROBB and H. CURTIS, Folia haemat. **44**:30, 1931.

The case reported concerns a man of 35 years whose blood and bone marrow showed a picture characteristic of acute leukemia. Although at the time of the first admission the patient was critically ill, within a short period he completely recovered. His blood picture too returned to normal and remained so for four and a half months. Such cases, according to the authors, would appear to indicate a non-neoplastic origin of the acute leukemia and would also seem to offer some hope for the ultimate curability of this disease. In acute leukemia there would seem to be a failure of the normal inhibitory factors controlling the growth of the white blood cells, or else a failure of those factors favoring cell maturation. In either event, disappearance of these perversions seems possible, and it is this potential reversibility that would make one so hopeful of the possibility of a cure, even though at present the factors that bring about favorable or unfavorable results are unknown.

B. M. FRIED.

FLUORESCENCE OF CANCER TISSUE. J. KOERBLER, Strahlentherapie **41**:510, 1931.

Exposing out surfaces of tumors of the breast to the light of an arc lamp with Goerz-Beck carbons, cancer tissue appeared on the photographic plate in sharp contrast to the surrounding normal tissue due to the fluorescence of the cancer tissue. But a similar effect was obtained in pictures of the wall of a hemorrhagic cyst of the breast and of a tuberculous granuloma of this organ. It is thought that the fluorescence is due to the presence of hematoporphyrin resulting from decomposition of hemoglobin by bacterial action or by metabolic (lactic acid) action of the tumor tissue.

WILHELM C. HUEPER.

METABOLISM OF TUMORS. S. EDLBACHER and W. KUTSCHER, Ztschr. f. physiol. Chem. **199**:200, 1931.

All rapidly growing tissues such as tumors, granulomas and embryonic tissue show a characteristic increase in the decomposition of arginine by arginase. Arginine phosphoric acid does not occur in mouse carcinomas. The production of inorganic phosphoric acid from carcinomatous pulp by treatment with bicarbonate is not inhibited by the addition of fluoride, while this takes place, when muscle tissue of normal and carcinomatous mice is treated in the same way. The production of phosphoric acid in tumor pulp is therefore not dependent on the glycolytic processes, but is probably due to the decomposition of phosphatids and nuclear

substances. Nucleic acid added to tumor pulp resulted in a marked production of inorganic phosphorus through the activity of a tumor phosphatase. Malignant tumors of mice, rats and man show this phenomenon in a striking fashion, while the dephosphorylizing process is slower and less extensive in benign tumors, granulomas, etc. The general metabolic effect of the tumor was demonstrated by the fact that animals with cancer show a marked dephosphorylizing reaction in the muscle tissue, which sometimes even surpasses that present in the tumor itself, while the reaction is negative in the muscle of normal animals. Tumor tissue is therefore not only characterized by an activation of the hydrolytic decomposition of carbohydrates, but also of that of arginine and nucleic acid.

WILHELM C. HUEPER.

CARCINOGENIC SUBSTANCES OF ROUS SARCOMA. E. FRAENKEL, Ztschr. f. physiol. Chem. **200**:126, 1931.

When the tumor agent of Rous sarcoma is adsorbed to trypan blue and isamine blue, no inhibition of "takes" is observed. The same results were obtained from elutions of "Hansagelb" and different types of thioindigo to which the tumor agent has been adsorbed. Inoculations were, however, negative, when the agent was adsorbed to "Lackorange." It is concluded that the Rous agent must be a chemical substance and not a virus.

WILHELM C. HUEPER.

SKIN CANCER IN RATS AFTER PROLONGED ULTRAVIOLET IRRADIATION. W. PUTSCHAR and F. HOLTZ, Ztschr. f. Krebsforsch. **33**:219, 1930.

By means of prolonged ultraviolet irradiation, the writers were able to produce skin cancers in the rat with some regularity. The shortest interval was twenty-seven weeks, but after thirty-seven weeks cancers were observed in all of six animals subjected to the irradiation. There were no apparent relations between the color of the animal and susceptibility to the light. The addition to the diet of irradiated cholesterol was without apparent effect. As concerns tissue changes, those of the connective tissues were purely degenerative, were a feature of the dermatitis and bore no evident relation to the onset of cancer. A specific effect of the irradiation was the periodic regeneration of the hair, with overgrowth of the woolly hairs and formation of giant hairs.

H. E. EGGERS.

THE ADRENALS IN RELATION TO TUMOR GROWTH. H. AULER and W. RUBENOW, Ztschr. f. Krebsforsch. **33**:292, 1930.

The effects were studied of the injection of various preparations of adrenal on the progress of implanted tumors in rats. In one series an extract of adrenal cortex was used; in a second series, this was combined with phenols or phenolic acids; and in a third, the animals were given injections of the products of the action of the extract on hydrochinone. Throughout there was observed a high percentage of tumor regressions, and similar results were seen after the injection of the ferment plus red cells, and with the use of a similar ferment derived from Aspergillus orycae. The effect is ascribed to the action of the adrenal ferment on polysaccharide metabolism; with one of their extracts the writers observed a tremendous activation of animal diastase and counteraction of the antiferment effect on this of tumor extracts. The activating material they believe to be of phosphatid character, and its counteractive effect they ascribe to combination with bodies of aldehyde nature.

H. E. EGGERS.

TOBACCO CANCER IN THE RABBIT. A. H. ROFFO, Ztschr. f. Krebsforsch. **33**:321, 1931.

By the daily application for eight months of a watery solution of the combustion products of tobacco, Roffo has succeeded in producing a skin cancer of

the rabbit's ear in one of ten animals. Solutions of nicotine and of the distillate from the residue after nicotine extraction were without effect. Roffo lists the possible active substances as essential oils, pyridine and empyreumatic substances, along with a content of potassium carbonate, bicarbonate and nitrate.

H. E. Eggers.

On the Causes of Death in Cancer. L. M. Schabad and R. W. Goriainowa, Ztschr. f. Krebsforsch. **33**:348, 1931.

This study of the changes that may be held directly responsible for death was made on a series of 932 autopsies in cases of cancer. Among these were 209 cases of cancer of the esophagus, 334 of the stomach, 82 of the intestines, 11 of which were primary in the small intestine, 52 of the liver and its adnexa, 87 of the lung and 168 of the uterus. Cancer of the breast was not included. With the cancers studied, metastases were found most regularly with cancers of the lung and liver, and least often with those of the biliary passages and the ampulla of Vater. Bone metastasis was observed in 2.6 per cent of all cases, most frequently with pulmonary carcinoma and that of the lesser curvature of the stomach. In 12 cases, splenic metastasis was observed, 5 of the growths being primary cancer of the lung. Two cancers, both of the colon, occurred in persons under 20; 36—4 of the esophagus, 9 of the stomach, 9 of the colon and 14 of the uterus—caused the death of persons under the age of 30; 13 per cent of all cases occurred in the decade between 31 and 40, with 17 per cent of all cases occurring in persons under this last age. The direct causes of death were in large part dependent on regional involvement, aside from terminal pneumonia, which was present in 20.7 per cent of all cases. Pulmonary gangrene was found frequently in connection with esophageal cancer, ascending infection of the urinary system with uterine and at times with colonic cancer, and suppurative peritonitis, which was the direct cause of death in 17 per cent of cases, in association with abdominal involvement either by the primary cancer or its metastases. Terminal hemorrhage was responsible in only 5 per cent; generalized carcinomatosis, in 20 per cent of lung cancers and in only from 2 to 4 per cent of uterine and esophageal cancers. Exhaustion was a very frequent cause of death in cancers of the esophagus and stomach, but was rare in other cancers.

H. E. Eggers.

Benign Tumors of the Sweat Glands. M. Glasunow, Ztschr. f. Krebsforsch. **33**:431, 1931.

There are here reported three cases of benign tumors of the sweat glands. The diagnosis is made on the criteria of traces of a double-layered epithelial wall, the presence of a definite basement membrane and of such accessory features as connection with more or less typical sweat ducts. Glasunow states that similar tumors are frequently reported in dermatologic literature under the designation of "Naevus epithelioma-cylindromatosus."

H. E. Eggers.

On Primary Carcinoma of the Liver. M. Solowej, Ztschr. f. Krebsforsch. **33**:442, 1931.

In a primary carcinoma of the liver reported here in a woman, aged 53, Solowej has found three fairly distinct stages of atypical growth: parts with a distinct preservation of organoid structure, those with marked disorder of cellular arrangement and those with pronounced abnormality of individual cells. The case showed tumor metastasis to the periaortal and periportal lymph glands and to the pleura. The author calls attention to a frequently present and somewhat important diagnostic feature: the absence of associated splenic enlargement, which, with the exclusion of such diseases as hepatic syphilis or echinococcus, suggests primary cancer in the liver.

H. E. Eggers.

CANCER MORTALITY IN THE UKRAINE. A. M. MERKOW, Ztschr. f. Krebsforsch. **34**:21, 1931.

Despite the fact that the death rate from cancer in the Ukraine has shown a steady increase during the limited period covered by this report, the present rate (1928) is still very low as compared with that of more western countries—69.4 per cent per hundred thousand. The explanation would appear to lie in the relatively preponderant early age distribution of the population. It would appear that in respect to death incidence in general, the Ukraine at present is in the same position as was western Europe a number of years ago, as there is still an unduly high death rate from infectious diseases, particularly tuberculosis and pneumonia. The statistics as to age distribution in deaths from cancer in the Ukraine show no essential differences from those obtained elsewhere; the maximum incidence occurs, as usual, in the sixth decade. As concerns deaths from cancer in persons under 50, these were more frequent in women; of these relatively early deaths from cancer in that sex, 50.8 per cent were caused from cancers of the genitalia and 42.6 per cent from cancers of the breast. In general, the usual age at which death from cancer occurred was higher in rural districts and in the smaller towns —a fact that the writer ascribes to the extensive migration of younger persons from those localities to the larger cities. This difference was not apparent with cancer of the genital organs and of the breast; indeed, with the former relationship was reversed.

H. E. EGGERS.

PRIMARY SARCOMA OF THE SPLEEN. K. SCIESINSKI, Trav. d. Inst. d'anat. path. d. Univ. de Cologne **2**:436, 1931.

A large round cell sarcoma of the spleen in a man 38 years of age is described. The origin of the tumor, which appears to represent a special group of sarcoma, is traced to the reticulum cells of the reticulo-endothelial system.

Medicolegal Pathology

SELF-MUTILATION OF DELINQUENTS. NUERNBERG, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:431, 1931.

For preventing imprisonment or for gaining certain advantages in penal institutions, delinquents not uncommonly inflict various injuries on themselves. Many kinds of artificially produced morbid conditions have been described and are briefly reviewed. Nuernberg cites fifteen new cases dealing with artificial phlegmon, subcutaneous emphysema of the face, swallowing of the broken handle of a spoon, insertion of needles beneath the skin, simulation of hemoptysis by suction of blood from the socket of a recently extracted tooth, production of acute otitis media by self-injection of aromatic oils, etc.

E. L. MILOSLAVICH.

COMPLICATIONS OF SYPHILITIC AORTITIS. R. POHL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:443, 1931.

In a period of two and one-half years, one hundred cases of syphilitic mesaortitis were observed, with great predominance of cases in males (87 per cent). In 21 per cent of the cases, the aortic leaflets were involved, and in 36 per cent hypertrophy of the heart was present, the weight of the heart reaching in half of the latter instances 500 Gm. or more. In 27 per cent, aortic aneurysm developed, and in half of the cases perforation resulted nine times within the pericardiac sac. Changes of the coronary arteries, especially stenosis of the main ostium, were observed in 13 per cent of the cases, and cardiac aneurysms were found five times. There is a certain predilection for occlusion of the proximal end of the right coronary artery. One case, involving a 28 year old woman with congenital stenosis of the isthmus of the aorta and diffuse syphilitic aortitis of the ascending portion, is described minutely.

E. L. MILOSLAVICH.

FATAL POISONING WITH THUJA OCCIDENTALIS. G. JUNGMICHEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:449, 1931.

In the literature of the last eighty years, only four cases of poisoning with Thuja occidentalis are recorded, the drug having been taken to produce abortion. An additional lethal case is reported, with the chemical and microscopic details. The autopsy disclosed a severe gastro-enteritis and cloudy swelling of all the parenchymatous organs, with beginning fatty degeneration. Extract of Thuja occidentalis causes contractions, cramps and congestion of the internal organs, but is not a specific abortifacient. Thuja occidentalis is more dangerous than sabina. In animal experiments with Thuja occidentalis, the liver shows a fatty degeneration of the central and peripheral zones of the acini.

E. L. MILOSLAVICH.

DIRECT FRACTURE OF THE BASE OF THE SKULL. B. PUCHOWSKI, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:487, 1931.

A male dwarf, 50 years old, was attacked and gored by a bull, the horn penetrating the left front side of the neck in an upward backward direction and entering into the skull laterally to the foramen occipitale magnum. Several ribs on the right side were found to have been fractured as a result of the impact of the animal.

E. L. MILOSLAVICH.

A PECULIAR INJURY OF THE SKIN DUE TO AN AUTOMOBILE ACCIDENT. P. HEILMANN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:490, 1931.

A honeycomb-like imprint was found in the skin at the left side of the forehead, due to impact of the radiator of an automobile. [A not uncommon occurrence in this country.]

E. L. MILOSLAVICH.

FATAL PARALDEHYDE POISONING. JULIUS BALÁZS, Samml. v. Vergiftungsfällen **2**:151, 1931.

In the case of a woman, 43 years old, who was found in coma, the diagnosis of paraldehyde poisoning was made mainly on account of the odor of paraldehyde on the breath and in the urine and then on the subsequent demonstration of the presence of paraldehyde in traces of the cerebrospinal fluid and in large quantities in the urine. The amount of paraldehyde taken could not be determined. After death, the mucous membrane of the fundus of the stomach was edematous and ecchymotic (from the open stomach came an intensive odor of paraldehyde); there was a moderate edema of the brain; the lungs were congested, and there were punctiform subpleural hemorrhages. These were the principal changes ascribed to the action of the paraldehyde. Only a few cases of fatal paraldehyde poisoning have been reported, most of them from England, and many of these cases are doubtful.

PHANODORN POISONING. K. WAGNER, Samml. v. Vergiftungsfällen **2**:159, 1931.

Phanodorn is the proprietary name for cyclohexenyl ethyl barbituric acid. The drug is used as a hypnotic. It is also called cyclobarbital. In the case of a man, 52 years old, who was found dead after having taken presumably a very large quantity of phanodorn some ten to eleven hours earlier, the postmortem examination failed to reveal any natural causes to explain the sudden death. Chemical examination revealed a small amount of phanodorn in the stomach, while 21 mg. of phanodorn was isolated from 100 cc. of urine. It appears that the organism rapidly destroys the substance, and its presence in the urine in this case is interpreted to mean that an unusually large amount had been taken.

VOLATILE POISONS. H. ZANGGER, Schweiz. med. Wchnschr. **61**:741, 1931.

In this and other recent articles in the same journal, Zangger describes new experiences and observations concerning volatile poisons, some of which are used as solvents for technical purposes in industry.

Technical

A DEVICE FOR CLEANING THE BOWEL POSTMORTEM. S. GRÖFF, Centralbl. f. allg. Path. u. path. Anat. **52**:97, 1931.

The author's device consists of a hollow horizontal bar, open on top so as to form a small trough. To the ends of this bar are attached forklike uprights to receive the lugs of a flat weight plate, convex on its lower edge to fit into the groove of the horizontal base bar. In operating the device, the bowel, removed from its mesentery and slit lengthwise, is drawn, mucosa uppermost, between the two bars so that the intestinal content is scraped off. A 300 Gm. weight plate 19½ inches (49.5 cm.) long is used for adult bowels and one weighing 150 Gm. for children.

G. RUKSTINAT.

THE GELATIN TEST FOR LIVER FUNCTION. RUDOLPH MANCKE, München. med. Wchnschr. **78**:1430, 1931.

This test is considered to measure the protein metabolism in patients with disturbances of the liver. It consists in the administration of a gelatin solution by mouth and the determination of amino-nitrogen in the urine at designated intervals. Under normal conditions, the maximum elimination is within the first four hours. In pathologic conditions, the total excretion may be increased or the spacial elimination changed.

AUTHOR'S SUMMARY.

AN IMPROVED METHOD OF OBTAINING RESIDUE STRUCTURES IN MICROSCOPIC SECTIONS BY DRY HEAT. O. SCHULTZ-BRAUNS, Verhandl. d. deutsch. path. Gesellsch. **26**:153, 1931.

Fresh frozen sections were heated in nitrogen in the drying oven at 530 C. After cooling, the sections were enclosed in balsam and examined by dark-field illumination. The structure of the tissue is well preserved in these preparations, and the distribution of the residues in the sections presents instructive pictures. The calcium content of normal and diseased heart, kidney, thyroid gland, brain and benign and malignant tumors is described. In amyloidosis, the deposits of amyloid contained always a considerable increase of residues, which consisted especially of calcium.

C. A. HELLWIG.

AN EXACT METHOD FOR THE DETERMINATION OF COAGULATION TIME. G. ELIAS, Ztschr. f. d. ges. exper. Med. **77**:693, 1931.

An exact method is presented which allows one to follow the progress of the clotting of blood and to represent it graphically. The method is based on the change in the Tyndall phenomenon (tyndallometric effect) which the transformation of fibrinogen into fibrin brings about. The beginning and end of the coagulation, so far as they represent the formation of fibrin, are definitely established in this manner. The method yields a reproducible curve. Such a method of investigation ought to lead to a deeper insight into the process of coagulation in general. The exactness of this method invites further studies concerning the change of fibrinogen into fibrin. With the newest type of photometer it was possible to carry out the foregoing experiments with 0.3 cc. of blood. For investigation of the human plasma this is a great advantage.

SANDER COHEN.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, Oct. 12, 1931

R. H. JAFFÉ, *President, in the Chair*

SKODA AND ROKITANSKY. R. H. JAFFÉ.

About a hundred years ago, morbid anatomy had very modest quarters in the General Hospital of Vienna. Its home was in a small building in one of the wide, tree-covered courts of the hospital. The room that served as pathologic-anatomic theater was just large enough to hold the wooden autopsy table and a few chairs. Most of the time artificial light was necessary, supplied by an oil lamp fastened to the ceiling by a string. In this room Johann Wagner, associate professor of pathology and prosector, gave his demonstrations. The room was packed with students and young physicians, who eagerly followed Wagner's autopsies and interesting demonstrations, since good lectures were scarce in the days of declining fame of the first great Viennese medical school.

Among the students who never missed a lecture in pathology were two shy young men who soon became friends. They had come to the big city from small Bohemian towns, and each was bashful because of the slight Slavic accent of his German. The younger one, Josef Skoda, the son of a blacksmith at Pilsen, had traveled to Vienna on foot. The older one, Carl Rokitansky, was better situated. Though his father, a government employee at Königgrätz, had died when Carl was but 8 years of age and had left the family in poor financial circumstances, an uncle offered some support, and Carl made the trip to Vienna in a peasant's wagon.

Skoda and Rokitansky had selected the study of medicine because of inclination. Full of enthusiasm, they soon recognized that morbid anatomy formed the basis for the understanding of the nature of diseases. It was the common interest in pathology which bound them together, and the acquaintance made in the small morgue soon grew into a friendship which was destined to give much to the science of medicine.

Though young in years, Skoda and Rokitansky were critical enough to recognize the deficiencies of the medical teaching of their days. In a letter to his relatives, Rokitansky called the lectures a "lot of words without real background." The whole conception of clinical medicine centered about symptoms, and little significance was attributed to the observation of the patient, the objective findings and the underlying organic lesions.

In their endeavor to find a better basis for their studies, Skoda and Rokitansky became acquainted with the achievements of the French school. Jean Nicolas Corvisart des Marest and Pierre Adolphe Piorry had revived and improved the method of percussion which had been described first by the Viennese physician, Leopold Auenbrugger, but which soon had been forgotten. The genial Theophile Hyacinthe Laennec had discovered auscultation and had correlated the acoustic findings with exact observations at the autopsy table. Compared with the dogmatic and somewhat mystical medicine that prevailed at this time, the new methods of the French meant a tremendous progress. These new ways became the starting point of Skoda and Rokitansky, who, however, did not simply apply and confirm what the French had found, but went much further, and their clear and critical minds deepened and supplemented what the esprit of the French had created.

After graduation, the pathways of Skoda and Rokitansky parted. Rokitansky stayed with Wagner at the morgue, first as an unpaid resident, later as a paid assistant. When Wagner died in 1832, Rokitansky substituted for two years,

and in 1834 was officially appointed as Wagner's successor. Ten years later, at the age of 40, Rokitansky was made professor of pathologic anatomy and head of the newly created department of pathology. Pathology was now a compulsory subject of the medical curriculum.

While Rokitansky never felt the inclination of making practical use of his knowledge at the patient's bedside, Skoda's field was in the hospital wards. In 1833 Skoda became resident physician in internal medicine at the General Hospital, in 1840 attending physician, and in 1846 head of the department of internal medicine. In his capacity as a clinician, Skoda remained a regular visitor of the morgue. By watching Rokitansky's autopsies and by correlating the anatomic findings with his clinical observations, Skoda learned his internal medicine. He had nothing else to teach him, except the book of the French authors. After the autopsies, he discussed his observations and conclusions with Rokitansky, and in these discussions Rokitansky usually was the donor. Virchow once called Rokitansky the first true descriptive pathologic anatomist, and compared him with Linné. This characterization, however, does not do full justice to Rokitansky, who questionably was more than a merely descriptive investigator, though he was the founder of the modes of expression in descriptive pathology. In his first publication dealing with the internal intestinal incarceration, Rokitansky described not only the anatomic changes, but also tried to explain the symptoms, and suggested therapeutic procedures. Many of his later publications revealed a similar trend. In later years, Rokitansky made himself familiar with the new science of histopathology, and he recognized that studies of morbid anatomy had to be completed by experimental observations on the living animal and by chemical research. When in 1875 Rokitansky resigned from the chair of pathology, he stated in his farewell address that his aim had been to do his work as pathologist in the spirit of a science the chief purpose of which was to stimulate clinical medicine. "As pathology has given to the knowledge of clinical medicine a broader and firmer foundation, it has itself penetrated into the pathologic histology. In order to complete itself through research on the living animal it has created experimental pathology."

After Skoda had empirically learned auscultation and percussion, he became much interested in the physical background of the variations of the sounds, and reconstructed artificial models in order to determine the conditions under which the different qualities of sound occurred. His talents in mathematics and physics helped in experiments which taught him that the classifications of qualities of sound given by the French were much too complicated and lacked a physical explanation. About the same time, a British physician, Latham, had reached similar conclusions.

The outcome of Skoda's studies was a monograph entitled "Treatise on Percussion and Auscultation," published in 1839. It took a long time before this booklet, printed on blotting paper, found the recognition that it deserved, for it undoubtedly belongs to the classics of the German medical literature. It is interesting to note that in this book the pulsation of the cervical veins and the accentuation of the second pulmonic sound are mentioned for the first time.

Though different in their fields, Skoda and Rokitansky reveal great similarity in their work. In both there is the tendency to simplify, to bring order into their respective sciences, to group similar conditions together and to separate what does not belong together. Both had an intuitive feeling for causal relations. Of the two, Rokitansky was the stronger personality. He guided Skoda in the right direction. Rokitansky's high opinion of Skoda is shown in his letter of recommendation written to the medical faculty on the occasion of filling the vacant chair of internal medicine: ". . . he (Skoda) is predestined as a teacher because of the brilliance of his mind, the correctness of his judgment, a man unbiased by the authority of others and by speculation, a shining light to the student, an example for the ambitious and a stronghold for the hesitating."

Skoda's and Rokitansky's new ways in medicine met with much opposition among the older members of the Viennese medical profession, who had been

educated in the spirit of the all-explaining symptomatology. The opposition merely strengthened the bonds of friendship. Skoda was Rokitansky's most ardent supporter, one of the first to recognize his greatness. But, in spite of the strong influence which Rokitansky exerted on Skoda, the latter did not give up his own judgment, and he did not follow Rokitansky where he was wrong. Thus, Skoda did not accept Rokitansky's theories of the dyscrasias in which he tried to trace the diseases to abnormal compositions of the blood, theories which were so severely criticized by the young Virchow. It was perhaps due to Skoda's influence that Rokitansky gave up his conception of the dyscrasias and came to the recognition that the organic lesions are the causes of the diseases.

In later years, Rokitansky, the philosopher, distinguished sharply between idealisms as a philosophy of life in the sense of Kant and Schopenhauer, and the strict materialism that should be the philosophy of research.

Rokitansky's greatest contribution to medical science was his textbook on pathology in three volumes, the first edition of which appeared in the years from 1840 to 1846. In the introduction to this first edition, Rokitansky stressed the importance of the materialism in science: "A right conception of power and matter teaches that there is no power without a substratum. Every phenomenon is the expression or the result of the matter which passes from one stage into another one." Rokitansky's textbook was based entirely on his own experiences, and he was unbiased by the preexistent great monographs on pathology by Morgagni, Baillie, Meckel and Andral.

Many other publications came from Rokitansky's tireless pen, such as those on: the spontaneous rupture of the aorta, the diverticulum-like dilatations of the trachea, the strictures of the intestinal tract, the penetrating gasric ulcer, the deformities of the spine and pelvis, the combination and exclusion of pathologic processes, the anatomy of goiter, the important diseases of the arteries, the papillary carcinoma, the growth of connective tissue and its relation to inflammation, the proliferation of connective tissue in the nervous system, and the defects of the septums of the heart.

Rokitansky's great interest in philosophic questions is shown in his two academic lectures: "The Independent Value of Knowledge" and "The Solidarity of Animal Life." In 1863 Rokitansky was called to the State Department of Education as adviser in questions of medical education. In this capacity he exerted a brilliant influence on the development of the medical faculties of Austria. He created special chairs for experimental pathology and medical chemistry, founded the first psychopathic clinic in Austria, called Billroth to Vienna and Klebs and Breisky to Prague. As a member of the Austrian senate, he urged the separation of the school from the church.

Skoda was a physician with all his heart. He divided his time between the clinic and his consulting practice. He wrote little, but what he wrote revealed his deep interest in pathologic anatomy. His greatness expressed itself in his lectures and bedside clinics. Ever and ever again he impressed on his audience the importance of morbid anatomy as the basis of clinical medicine, and he considered his teaching as part of Rokitansky's work. His clear and simple diction enabled him to transmit to his pupils his trends of thought, and every demonstration of a case not only afforded a wealth of information, but was a great stimulus to independent thinking. Instead of writing papers and books, Skoda became the founder of a great school of clinicians famous for their keenness of observation and correctness of diagnosis, which has often been called the pathologic anatomic school of medicine. I do not know of a better example of the great progress that can be accomplished by the close cooperation between clinician and pathologist than is revealed by the lives of Skoda and Rokitansky.

DUAL CARCINOMAS, PRIMARY IN THE LUNG AND IN THE PROSTATE. P. A. DELANEY.

A white man, aged 64, had a primary carcinoma of the lung. The postmortem examination demonstrated an extensive carcinoma of the left lung, the cells of

which were small and formed no regular structural units. The metastases were widespread and extensive. A second carcinoma was found in the prostate; the tumor infiltrations also were anaplastic cells.

COMBINED CONGENITAL EXSTROPHY OF THE FEMALE URINARY BLADDER AND CLOACA. GRAHAM A. KERNWEIN.

The report will be published in the ARCHIVES.

TULAREMIC LEPTOMENINGITIS. ARTHUR R. BRYANT and EDWIN F. HIRSCH.

The report was published in the ARCHIVES (**12**:917, 1931).

Regular Monthly Meeting, Nov. 9, 1931

R. H. JAFFÉ, *President, in the Chair*

THE ANTIGENIC RELATIONSHIP OF THE LIPOIDS FROM BRAIN AND TESTICLE. JULIAN H. LEWIS.

Rabbits immunized with emulsions of beef testicle produce antiserums that react with the alcoholic extract of testicle and brain. They do not react with the alcoholic extracts of the other organs. Positive reactions are obtained with the brain and testicle of every species tested. Antibrain and antitesticle serums react with cholesterol, while antiliver and antikidney serums do not react with this substance. None of the antiserums reacted with lecithin.

HEMOCHROMATOSIS AND PRIMARY CARCINOMA OF THE LIVER. S. R. ROSENTHAL.

The article was published in the ARCHIVES (**13**:88, 1932).

BILATERAL DIFFUSE CORTICAL NECROSIS OF THE KIDNEYS. A. WOLFSON.

Rare instances of bilateral diffuse cortical necrosis of the kidneys, occurring in the absence of thrombosis of the larger renal vessels, have been reported from time to time. Most of the thirty-five reported cases occurred with pregnancy, but approximately ten occurred with scarlet fever, diphtheria, dysentery, etc. The first case of which I have found any record was reported by Friedländer in 1883 and occurred in a 36 year old woman convalescent from scarlet fever.

A colored woman, 35 years of age, a sextipara, was admitted to a medical ward of the Cook County Hospital on April 28, 1931. She died within a few minutes after reaching the ward. The diagnosis in the examining room was pneumonia. Three months before admission, the patient, then about three months pregnant, had weakness which became progressively worse. Two weeks before admission, she began to have severe headaches and emesia. Two days later, or three days before admission, she had vaginal bleeding. A physician terminated the pregnancy. The symptoms became markedly worse. She had almost complete anuria, which persisted until death. During this time only small quantities of turbid, bloody urine were passed. Two days after the onset of the anuria, she had spots before the eyes and blurred vision, which lasted until death. There were also chills and fever, a foul-smelling vaginal discharge and dyspnea. The patient was conscious until death, which occurred rather suddenly.

The postmortem examination was performed by Dr. Jaffé. The most striking findings were in the kidneys. Other changes were hypertrophy of the heart (weight, 550 Gm.), postpuerperal uterus with pseudomembranous endometritis (Staphylococcus in cultures) and focal necrosis of the liver. The indican content of blood obtained at autopsy was approximately 5 mg. per hundred cubic centi-

meters. There was a peculiar localized cortical necrosis of the kidneys in intimate relation with certain segmentary vascular changes affecting particularly the interlobular and arcuate arteries. The interlobar and larger renal vessels were almost entirely free. The glomerular tufts and the afferent vessels as well as the interlobular arteries were widely dilated and filled with erythrocytic débris, and most of the interlobular vessels with thrombi. The arcuate arteries were patent, but many had marked intimal proliferative changes and advanced organizing thrombi. The medullary tissue and the marginal zones of cortical tissue were well preserved. In relation to these zones, toward the necrotic regions, was extensive calcification of the tubules and glomeruli. Not all blood vessels had the same changes. Some of the interlobular arteries distally were distended and contained fibrin and fairly intact erythrocytes. In relation to these vessels were islands of tissue with distinct tubular and glomerular details. In other regions the entire interlobular artery and even the associated arcuate artery were extensively thrombosed. Where arcuate vessels were thrombosed they had the most extensive organization. The latter type of interlobular artery was in relation to those areas of cortex that had the most severe necrosis.

Bacterial stains failed to reveal the presence of organisms in the tissues.

THROMBOSIS OF THE HEPATIC ARTERY WITH SUDDEN RESISTANCE TO INSULIN IN A DIABETIC PATIENT. HERBERT POLLACK AND ESMOND R. LONG.

I. K., a white man, 56 years of age, was diabetic for nineteen years and under dietary control. In 1924 he had a midthigh amputation for a gangrenous left foot. He first came to the University of Chicago Clinics in January, 1931, with a grangrenous toe on his right foot. Supracondylar amputation was done, depriving him of his remaining leg.

He was observed frequently in the out-patient department and once in the hospital for two days. On June 15, 1931, he was admitted with the complaints of vomiting, some abdominal pain and persisent sugar in his urine. Physical examination revealed only marked acidosis. The blood sugar was 410 mg. per hundred cubic centimeters. The urine contained much sugar and acetone. The first six days he received from 90 to 130 units of insulin and was brought out of acidosis. On the seventh day a mild acidosis suddenly developed, and 470 units of insulin was given. The acidosis was improved, but the blood sugar remained between 200 and 400 mg. per hundred cubic centimeters. The next day he received 540 units of insulin, and the blood sugar became 184 mg. per hundred cubic centimeters. Because of an enlarged thyroid gland and the resistance to insulin, compound solution of iodine was given by rectum the following day. Four hours later the blood sugar fell to the levels found in a state of shock. Three hundred grams of dextrose was given intravenously, subpectorally, orally and by proctoclysis before the blood sugar rose to 54 mg. per hundred cubic centimeters. It continued to rise in spite of the administration of 260 units more of insulin.

The next morning the patient was sitting up in bed and was rational. A definite odor of acetone was noticeable on his breath. The blood sugar was too low to be estimated at this time. In spite of forced administration of dextrose, he died in a few hours.

The necropsy findings, as reported here, were unexpected. While mesenteric thrombosis was diagnosed, the gangrenous pylorus with perforation and peritonitis were not suspected. The extensive thrombosis of the vessels arising from the celiac artery was another surprise.

The most significant lesions disclosed by the necropsy were: advanced generalized atherosclerosis; arteriosclerotic atrophy of the pancreas; recent thrombotic occlusion of all branches of the celiac artery; necrosis of the wall of the lesser curvature of the stomach; acute generalized peritonitis; thrombotic occlusion of the pancreatic branches of the hepatic artery and recent anemic necrosis of a considerable portion of the pancreas; multiple recent anemic infarcts of the liver; recent and encapsulated anemic infarcts of the spleen; advanced atherosclerosis

of the coronary arteries of the heart, with recent infarction of the myocardium of the left ventricle; arteriosclerotic atrophy of both kidneys; recent thrombosis of the right renal artery; recent anemic infarct of the right kidney; marked atherosclerosis of the arteries of the leg stumps and organizing thrombus of the right common iliac artery and its branches; mural thrombosis of the first part of the thoracic aorta; colloid goiter; hyperthrophy of the prostate; hypertrophy of the wall of the urinary bladder; slight acute hypostatic bronchopneumonia on the right.

The condition of the celiac artery and its branches is illustrated in the accompanying diagram. The splenic, left gastric and hepatic arteries were thrombosed, and the rounded end of the thrombus projected into the celiac artery. The splenic artery was a dilated, tortuous cord, thrombosed throughout. The left gastric and gastro-epiploic arteries were thrombosed in their beginning portions, but not in the distal parts, which were presumably patent because of anastomoses with branches from the middle colic and esophageal arteries. The hepatic artery and all its branches (right and left hepatica propria, right gastric, gastroduodenal and an accessory pancreatic artery) were thrombosed throughout their length. The

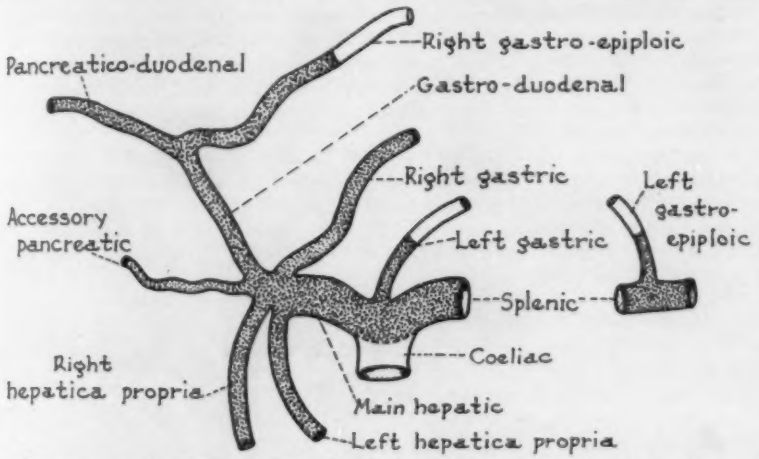

Diagram to show the thrombosis of the celiac artery and its branches.

pancreaticoduodenal artery was completely occluded, but the right gastro-epiploic, like the left gastro-epiploic and left gastric arteries, was thrombosed only in its beginning portion, distally being patent by anastomoses with other vessels. The right gastric (pyloric) artery was thrombosed throughout its length. It led directly into a gangrenous region, 7 by 13 cm., in the pylorus and pyloric portion of the lesser curvature of the stomach. Spontaneous perforation of this resulted in fatal peritonitis.

The pancreas weighed only 55 Gm. Large portions were necrotic. The arteries in the substance of the gland were hard. Microscopically, there was extensive arterial thrombosis with necrosis of the gland substance and leukocytic reactions around the necrotic regions. The arteries in general were thickened, and the fibrous tissue was increased throughout the gland. Hyaline changes were present in many of the islets of Langerhans.

The liver weighed 1,800 Gm. In the dome was a discolored, yellow region 6 by 8 cm. at the surface and of slightly softer consistency than the surrounding hepatic tissue. Other smaller areas of similar changes were scattered throughout the liver. Microscopically, early degenerative changes were seen in the hepatic cells of these regions, including loss in nuclear staining and swelling and vacuolization of the cytoplasm. Few leukocytes were present, and the affected areas were

not demarcated. The cellular glycogen of such regions was abundant and apparently not different from that observed normally. The gallbladder was connected by adhesions with the right colic flexure and appeared in no way modified by the thrombosis of the hepatic artery.

Regular Monthly Meeting, Dec. 14, 1931

R. H. JAFFÉ, *President, in the Chair*

THE RÔLE OF NUTRITION AS A FACTOR IN THE PREVENTION AND TREATMENT OF DENTAL DISEASES. M. T. HANKE, C. M. MARBERG and W. H. TUCKER.

Data obtained by numerous investigators indicate that changes in the structure of the teeth may be associated with diets deficient in vitamins. Changes in dental structure are obtained in young animals when they receive diets deficient in either vitamin D or vitamin C. A demonstration of the histologic changes produced in the teeth and in the surrounding structures when guinea-pigs are fed diets deficient in vitamin C was made by means of natural color photographs of the sections. Experiments on guinea-pigs also show that rapid repair of the injured tissues occurs when the scorbutic animals are given orange or lemon juice in addition to the basal diet.

A survey of the literature and a study of 400 private patients conducted in collaboration with the Chicago Dental Research Club indicate that most persons are ingesting diets that are deficient in vitamins and, possibly, in other constituents. Diseases of the oral tissues, such as gingivitis, periodontitis or dental caries, are most frequently found in persons whose diets are markedly deficient in one respect or another. A deficiency in vitamin C appears to be most common. A deficiency in vitamin D is not uncommon. Usually, then, one is confronted with at least a dual deficiency. On the assumption that many of the pathologic dental conditions are in some way associated with nutritional deficiencies, we have, for three years, been advocating a very liberal diet that should contain all of the constituents that are essential for the development of healthy body tissue.

On this dietary regimen, gingivitis that is not associated with calculus disappears fairly rapidly (in from thirty to sixty days) in most cases. Calculus must, of course, be removed in any case if a complete recovery is to be obtained. Loose teeth frequently become tight. Alveolar bone is not regenerated. A large proportion of the pyorrheal alveoli have become free from pus, and most of the loose teeth have become satisfactorily tight. This takes time; in some cases, as long as two years. Teeth that are very loose, owing to a loss of alveolar bone, do not become tight and can hardly be kept free from pus.

About 85 per cent of patients who had formerly been afflicted with rapid progressive decay have remained free from dental caries for from two to three years. Some patients have been refractory, and in some the amount of caries has decreased, but the process has continued. Nutritional deficiency is probably not the only cause of dental caries; but it appears to be an important factor.

We have never observed a case in which a spontaneous recalcification of a carious lesion has occurred. Although we have numerous cases in which the cavities have not become larger, we have no cases in which the cavities have become smaller.

These preliminary findings have been subjected to a careful recheck under controlled conditions on a group of 422 children at Moosehart, Ill. The children ranged in age from 10 to 17 years—323 regular children and 99 controls. The 323 regular children were observed for one year on the standard Moosehart diet, which was somewhat deficient in vitamins A and D and quite deficient in vitamin C.

All but the small approximal cavities were filled at the beginning of this period. Gingival conditions were recorded by means of natural color photography. We

also obtained fourteen film dental roentgenograms on all children (at twelve month intervals), determined the calcium content (total and ultrafiltrable) and the total phosphorus content of the blood serum (at six month intervals), determined the hydrogen ion concentration in various regions in the mouth, studied x-ray pictures of the wrist (at twelve month intervals), made a study of the bacterial flora of the mouth, and determined the weights and heights of the children at six month intervals.

During this control period, 70.5 per cent of the children had some form of gingivitis; in 80.83 per cent carious lesions developed.

After one year on the standard diet, the cavities were again filled. No other dental work was done. The standard Moosehart diet was then augmented by the addition of 16 ounces (473 cc.) of orange juice and the juice of 1 lemon per day (this is the test period).

All of the gingivitis that was not associated with calculus disappeared during the one year test period (citrus fruit juice). In some of the cases with calculus great improvement occurred, and in some the condition healed entirely, in spite of the calculus. In most cases, however, the presence of calculus effectively prevented improvement.

Dental caries was arrested to the extent of 50 per cent and retarded to the extent of 16 per cent. We consider caries to have been arrested if cavities developed during the one year control period and did not develop during the one year test period.

This shows that the carious process can be influenced by some constituent of orange and lemon juice in addition to the standard diet. This constituent may be vitamin C, but our work does not prove this. The diet is not as adequate as that which we have been advocating to private patients; the results were not as good. This fact may be very important.

The boys at Moosehart show a gain in weight and an increase in height that are below the normal standards in some age groups (standard Moosehart diet). The addition of orange juice to the diet led to an increase in weight and height such that the values, for this one year period, were often in excess of the normal standards. Orange juice therefore supplied something that materially enhanced the rate of growth of the boys. This increase cannot be attributed to the slight increase in the number of calories ingested, because the children at Moosehart received an adequate number of calories.

The relation of total weight to height is approximately correct in all groups even on the standard Moosehart diet.

The determinations of the calcium and phosphorus of the blood serum which were conducted at six month intervals on every child have shown that (1) the values are all within the normal range; (2) the values obtained in the spring are slightly lower than those in the fall, which may indicate a deficiency in vitamin D, and (3) the values for total and ultrafiltrable calcium and for total phosphorus are no different in the group in which carious lesions developed than in the group in which these lesions did not develop. The development of carious lesions is not associated with deviations in the calcium and phosphorus content of the blood serum.

We have made a careful study of the occurrence of aciduric micro-organisms (that are also acidogenic) in the mouths because of the prevalent belief that carious lesions are initiated by the decalcifying action of acids, which, in turn, are produced by the metabolic action of bacteria on carbohydrates. Three groups of such micro-organisms were demonstrated, namely: streptococci, which were almost always present; Lactobacillus acidophilus, present as discussed later, and yeast, which was present in about 33 per cent of the mouths. L. acidophilus appeared consistently in the mouths of some children that were not afflicted with dental caries. This micro-organism is not, therefore, an obligate producer of dental caries. This statement is equally true of streptococci. L. acidophilus was found most frequently and consistently (but not invariably) in mouths that contained numerous carious lesions.

The accumulation of food débris in cavities may provide conditions that are favorable for the localization of this micro-organism in the cavities.

Our bacteriologic studies have done nothing either to prove or disprove the theory that dental caries is the result of the action of bacterial products on the dental substance.

Dental caries is most apt to occur in mouths of children who have had considerable caries in the past. Filling the old cavities does not, apparently, prevent the development of new cavities. A definite tendency toward dental caries exists, which appears to influence the progress of the disease during adolescence. We might consider this tendency to be due to heredity. This is a proper consideration if we enlarge the definition of heredity to include a varied ability to remain healthy in the face of adversity. Since all of the children at Moosehart are living under identical conditions, the existing state of deficiency, dietary or otherwise, will affect some children more unfavorably than others only if certain children require more of a given thing, or things, to remain healthy than is required by others.

It has long been claimed that the teeth decay most rapidly during the period of puberty. This is, indeed. the case; but the increase, during the period of puberty, is hardly as great as one would have expected.

An improvement has occurred during the test period in all groups irrespective of any previous tendency toward dental caries or of age. The addition to the diet of this one constituent has in many cases overcome the untoward effects of heredity and of puberty. This would strongly suggest that the citrus fruit juice contains something with which these children have previously been insufficiently supplied. The quantity of citrus fruit juice administered was so large that it proved adequate even for those who showed the greatest evidence of deficiency.

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, Dec. 10, 1931

LEILA CHARLTON KNOX, *President, in the Chair*

SARCOMA-LIKE TUMOR OF THE PLEURA. MENDEL JACOBI and HERMAN BOLKER.

A white boy, 6½ years of age, with a history of rheumatic fever and rheumatic mitral disease, had had for several months cough, intermittent fever and a suggestive bulging of the left upper part of the chest anteriorly. A diagnosis of pleurisy with effusion was made. At autopsy, a large mass was present in the left side of the chest, completely covered by visceral pleura, adherent to parietal pleura, and compressing the left lung against the vertebral column. The center of the mass was necrotic and hemorrhagic. It was composed of irregular groups of round and spindle-shaped cells. Definite invasion of blood vessels was shown. There was no invasion of pulmonary parenchyma. The heart presented a chronic and subacute rheumatic endocarditis, with Aschoff bodies. The pathologic diagnosis was: sarcoma of the pleura, visceral, subserous type, and rheumatic pancarditis.

DISCUSSION

PAUL KLEMPERER: These tumors are interesting because of the type of cell concerned. In the group of cases which Dr. Rabin and I described a year ago we were dealing mainly with the spindle cell type of sarcoma, except for one case in which there was an embryonal type of connective tissue present. These round cells in general suggest a more malignant type of tumor. This might account for the invasion of tissue in this instance. I might say, however, that one of our cases which at the first examination presented the picture of a typical

fibrosarcoma showed in its latest stages, at an operation performed about four years after the first one, a marked variation of the type of cell, and in this instance there were also metastases found within the other lung four years after the first operation, at which the entire tumor could not be removed. It is remarkable that even with these metastases the patient is still living today, which indicates that tumors arising from the subpleural tissues are not as malignant as other types of tumors, particularly not as malignant as pleural tumors that originate from the mesothelium. The case is unique because of the age of the patient. Generally these tumors occur at a much later stage of life.

ALFRED PLAUT: May I ask how the reticulum of this tumor looks? Have you used any silver stain or Mallory's stain?

HERMAN BOLKER: We have not used a silver stain. There was little fibrous tissue surrounding the tumor. Where the cells were in groups there were no fibers.

ALFRED PLAUT: It might be interesting to try a silver stain.

MYCOTIC ANEURYSMS AT THE ROOT OF THE AORTA IN SUBACUTE BACTERIAL ENDOCARDITIS. DAVID PERLA and (by invitation) SAM ROSEN.

An instance of mycotic aneurysms in the sinuses of Valsalva in a case of subacute bacterial endocarditis (Streptococcus viridans) was reported. A boy, aged 17, presented the clinical and pathologic findings of an old rheumatic lesion of the aortic and mitral valves with aortic insufficiency, mitral insufficiency and embolic lesions in the kidneys, brain and intestinal tract. The pathogenesis of the mycotic aortic aneurysms in subacute bacterial endocarditis was discussed. Thrombosis by embolization of small branches of the coronary arteries supplying the root of the aorta is followed by anemic necrosis and septic infarction of the wall of the aorta, with subsequent abscess formation. Pouching of these areas occurs with rupture and the production of aneurysms by dissection through the necrotic area.

In the instance reported in this paper, embolic thrombosis of vasa vasorum was followed by a periaortitis and mesaortitis with abscess formation in the adventitia and media and subsequent rupture into the lumen of the aorta. The presence of organized thrombotic occlusion of vasa vasorum with recanalization outside the wall of the old aneurysm further suggests the embolic origin of the lesion.

DISCUSSION

IRVING GRAEF: I should like to ask if Dr. Perla feels that antecedent rheumatic aortitis has been ruled out as an underlying basis for the development of some of these aneurysms in subacute bacterial endocarditis.

DAVID PERLA: It may be that healed rheumatic lesions in the aorta, particularly the intimal lesions, predispose to the implantation of vegetations on the intima of the aorta from the lesions on the aortic valve in subacute bacterial endocarditis. In this case, a small, healed intimal rheumatic lesion a few centimeters above the aortic cusp was noted, but no others were observed. I do not believe that rheumatic infection played any direct rôle in the formation of the aneurysms.

STUDIES ON THE HISTOLOGIC DISTRIBUTION OF FAT AND LIPOIDS IN VARIOUS DISEASES OF THE KIDNEY. A PRELIMINARY REPORT. IRVING GRAEF and (by invitation) HENRY HORN.

An analysis is presented, based on histochemical examination, of the distribution of fat and lipoids in various lesions of the kidney. The classification adhered to is that of Volhard and Fahr. The final pathologic diagnosis of the renal conditions is based on study of sections stained with hematoxylin and eosin, by van Gieson's and Weigert's elastica methods, with McGregor's modification of

Mallory's connective tissue stain, and by Heidenhain's azan carmine methods. Tissues of patients succumbing to acute infections and of patients dying in congestive heart failure have also been studied. The tissues of the kidneys, lungs and liver have been examined in each of ninety-nine cases, that of the kidneys with special reference to the distribution of fat and that of the lungs and liver for signs of stasis. Edema and ascites have also been looked for. This has been done to correlate signs of stasis with deposition of fat in the kidney.

For the study of fat, staining with sudan III counterstained with hematoxylin, treatment with osmic acid, staining with Nile blue sulphate and use of the polariscope have been the methods employed. Several frozen sections of formaldehyde-fixed tissue from each case were so studied.

In four of the eight normal kidneys studied, scanty intracellular deposits were found in the distal collecting tubules. There were no signs of stasis in either the lungs or the liver. Eight cases of congestive heart failure, six with advanced rheumatic valvular disease, one with severe coronary sclerosis and one with syphilitic aortitis, are included. In each were found marked signs of stasis, and in each, prominent fatty deposition in the epithelium of all varieties of tubules. Four cases in young adults, in whom an acute infection was the cause of death, presented prominent fatty deposits in tubules of all types associated with signs of stasis in sections of the liver and lungs.

One case of acute, diffuse glomerulonephritis with minute glomerular deposits revealed prominent fatty deposits in the tubules. There were marked signs of passive hyperemia in the liver and lungs.

In eleven cases of subacute, diffuse glomerulonephritis the vessels showed slight change. In ten, which were of the extracapillary form, the crescents always contained more striking deposits than the tufts proper. Nine of the eleven revealed prominent tubular fatty change. In seven of these there were associated definite signs of stasis elsewhere. Only two of the group with moderate glomerular deposits and signs of stasis showed slight tubular alteration. In the remaining two cases, tubular changes seemed commensurate with the glomerular alterations.

Of seven cases of chronic, diffuse glomerulonephritis, three in which the kidneys weighed more than 250 Gm. together revealed slight vascular deposits. In the four in which the kidneys weighed less than 250 Gm., the vascular deposits were more marked, in some instances entirely obscuring the lumen. The glomerular deposits were more prominent than in the cases of subacute nephritis. In only three of the group were tubular deposits commensurate with the changes in the other units. These cases also presented definite signs of stasis in the lungs and liver. In two cases with contracted kidneys there were slight tubular deposits even in the presence of marked vascular and glomerular change. In these, the signs of passive hyperemia were equivocal. In the remaining two cases with moderate vascular and glomerular deposits and slight focal tubular changes, the evidence of stasis was scanty.

The nephrosclerotic group consists of forty-three cases comprising two groups. The first group includes kidneys weighing together between 250 and 350 Gm.; the second group, kidneys weighing between 100 and 250 Gm. In the first group, nineteen specimens were examined. Fatty deposits were found in all units—consisting of subendothelial deposits in the afferent arterioles and of basal intimal deposits in the interlobular branches, and in the glomeruli varying from fine to globoid intracellular and extracellular masses of fat. In ten of the nineteen, the tubular deposits were far more prominent than the vascular or glomerular changes, and in all ten there was prominent evidence of stasis. In two, in addition, there were edema and ascites. In the remaining nine cases, only one instance with slight tubular deposit revealed definite signs of hyperemia. The remainder presented tubular changes corresponding to the alteration in the other units.

In the fourteen cases with kidneys weighing less than 250 Gm. together, the fatty deposits were more marked. An apparent association between passive hyperemia and tubular deposition was also observed here.

In four cases of the so-called end-stage of nephrosclerosis were found marked glomerular and vascular deposits and but slight tubular deposits. In three of the

four there were slight signs of stasis. In the fourth case there was slight pigmentation of the central hepatic cords. There was no edema in any instance.

In six cases of malignant nephrosclerosis, the accelerated form of Löhlein and Klemperer, marked fatty deposits were found in all units, contrary to the belief of Herxheimer. In every instance there were striking and widespread signs of stasis in the liver and lungs. In two there were edema and ascites.

In seven cases of focal embolic glomerulonephritis with striking deposits in the epithelium of all varieties of tubules, there were definite signs of passive hyperemia. In four, there were edema and ascites. Five cases of amyloidosis presented slight vascular and glomerular deposits and moderate tubular deposits. In three, there were definite signs of passive hyperemia. In one case with scanty deposits, there were no signs of stasis. The fifth case with marked fatty deposits in all units showed only slight signs of passive hyperemia.

Four cases of mercurial nephrosis showed scant focal globular deposits close to regenerating cords of tubules. The associated signs of stasis were slight. A fifth case with antecedent arteriolonephrosclerosis and signs of passive hyperemia had marked tubular deposition of fat.

One instance of acute interstitial nephritis occurring in pneumococcal pneumonia presented slight vascular internal glomerular deposits. Tubular epithelial deposits were frequently encountered in the cells of the collecting tubules. There were signs of congestion in the liver and lungs.

We have found sudan III combined with hematoxylin a satisfactory stain for fat and lipoids; Nile blue, sulphate, very irregular and inconsistent, and valueless in older material; examination by means of the polariscope showed doubly refractile substance in the chronic nephropathies, usually scant in amount and invariably in association with sudanophil material.

Fatty substances have been found in a wide variety of conditions. While fat phanerosis can explain histologic increase or appearance in organs showing no chemical increase, as Wells has stressed, passive hyperemia ocurs in many patients with moderate or severe nephropathies and must be taken into account in explaining the appearance of fat in uriniferous tubules.

DISCUSSION

PAUL KLEMPERER: While I think that one must agree that passive congestion, particularly in heart failure, is very frequently or almost always associated with a marked fatty infiltration of the renal tubules, so much so that we speak of the characteristic appearance of the kidney in heart failure, I should like to ask Dr. Graef whether he believes this is the only cause for deposition of fat. I do not think that one should generalize, because there is a marked fatty change in mushroom or phosphorus poisoning, which hardly can be explained on the basis of passive congestion. I think we have to consider other reasons, particularly metabolic causes, for the deposition of fat in these kidneys.

IRVING GRAEF: I should like to make only two points in addition to what Dr. Horn has said. In the case of benign nephrosclerosis with heart failure it is striking that the tubular deposition of fat is just as marked in the intact tubules as in the atrophic tubules. Many atrophic tubules shed their lining epithelium after going through the stage of deposition of fat. The more severe arteriolar lesions are undoubtedly associated with a degree of tubular injury that must be related to the etiologic factor, which is as yet unknown, but in these cases, too, the degree of heart failure is so striking that the association cannot be disregarded. In the amyloid kidney there is a totally different picture. In our material, three cases were associated with signs of passive hyperemia. Of these, two presented edema and ascites. The mechanism for this edema and ascites, we recognize, may be related to a special dysfunction of the kidney somewhat analogous to true lipoid nephrosis. In the fourth case with signs of passive hyperemia, we found practically no fat in the tubules. In the fifth case with no signs of hyperemia, we found abundant fat.

The study is still in progress, and we should be glad to receive any suggestions. We are particularly disappointed in the use of Nile blue sulphate. We found it a most unreliable dye, and we could not depend on the results, particularly since the aging of the material makes considerable difference in the consistency of the results.

ENDEMIC AMEBIC DYSENTERY IN RESIDENTS OF NEW YORK CITY. PAUL KLEMPERER.

Eleven cases of amebic dysentery observed within the last five years were reported. Five of the patients were foreign-born, and probably had contracted the disease in their native country, though two of them had been residents of New York City for the last four and eight years, respectively. The other five patients were natives of the United States and had lived all their lives in or around New York City; one of these patients had been a resident of Missouri. The last patient was born in Austria, but had been living in New York City for the past thirty years. Only in four of the eleven cases was a clinical diagnosis of amebic dysentery confirmed by examination of stools. In the other seven cases, the condition was revealed only at the autopsy table or microscopically in surgical specimens removed from the patient. The clinical diagnosis in these cases was carcinoma of the large intestine in three instances, peritonitis, acute appendicitis and ulcerative colitis each in one. One patient who died from bilateral lobar pneumonia showed amebic dysentery as an incidental finding.

DISCUSSION

LOUIS FAUGERES BISHOP, JR.: In a study of amebiasis, sixty cases occurring at Bellevue Hospital were received. About twenty-five per cent of the patients had no history of ever having been out of New York City, so far as could be determined. For that reason I feel as Dr. Klemperer has said, that amebiasis is probably far more frequent in New York City than any of us who are practicing here realize. It is not always an easy matter to make a diagnosis, and I think Dr. Klemperer has brought out the fact that all of us should be more on the lookout to make the diagnosis of amebiasis before autopsy. I think with a more careful examination of stools, using the warm stage, we shall be able to distinguish these cases earlier, and by so doing, be able to help these patients.

W. GRETHMANN: I think Dr. Klemperer is justified in stressing the necessity of looking for patients with Entamoeba histolytica. In the tuberculosis wards of Bellevue Hospital in the course of the last year I had an opportunity to see three cases of entameba infestation. All three patients, as far as I can remember, were residents of New York and there was nothing in their histories to reveal that they had lived in the South. The first case was combined with tuberculosis; intestinal symptoms were present. The latter were naturally attributed to tuberculosis, and the presence of Entamoeba histolytica was not suspected clinically, but was found post mortem. Soon after this there was a second case in which the pulmonary lesion and the picture as a whole did not seem to indicate so severe an intestinal lesion as the patient showed clinically; the feces were searched for Entamoebae, and they were found. The third case was that of a patient suffering apparently from an abscess of the lung. Operation was done, and it was found that the abscess was too large for successful drainage and the wound was closed. At autopsy, the large intestine was considerably involved by ulcerated lesions, but by far the larger lesion was found in the liver. Practically the whole of the dome of the liver was transformed into a crater-like lesion measuring about 15 cm. in diameter. There were direct ulceration and penetration of the diaphragm and empyema on the corresponding side, together with ulceration of the lower lobe of the right lung. I think the three cases serve to substantiate Dr. Klemperer's opinion that infestation with Entamoeba histolytica is not uncommon in New York.

Book Reviews

Studien über die Entstehung und den Verlauf der Lungenkrankheiten. By Dr. N. P. Tendeloo, o. ö. Professor der allgemeinen Pathologie und der pathologischen Anatomie, Direktor des pathologischen Instituts der Reichsuniversität Leiden. Second edition. Paper. Price, 26 marks. Pp. 219, with 6 illustrations. Munich: J. F. Bergmann, 1931.

Of the first edition entitled "Studien über die Ursachen der Lungenkrankheiten," the "Physiologischer Teil" with a separate bibliography was published in 1900; the "Pathologischer Teil" was published two years later; the completed work was dedicated to van Heukelom. At this time the author was prosektor in the municipal hospital at Rotterdam. For a number of years he has been professor of pathology in the university at Leiden and is well known through his textbook "Allgemeine Pathologie," first published in 1919, with a second edition in 1925. These studies of pulmonary disease, revised after about thirty years, are so completely rewritten that only traces of the original text remain. A new format with smaller modern type, larger printed space, some condensation and the total pages cut to less than half of the first edition have all combined to produce an attractive monograph. Except for slight rearrangement and new captions for some chapters and minor subdivisions, the plan of the original work is closely followed.

The physiologic studies are of forces responsible for the capacity of the lungs; modifications resulting from localized dilation or compression; variations in different parts of the lungs of their normal respiratory capacity; energy of air currents, the blood and lymph flow in the lungs, and finally, practical applications of these with especial attention to their relation to the inhalation of dust and to drowning. Discussion of the mechanics of normal respiration, of the ease of movements involved in quiet breathing and the tonus and relaxation of respiratory muscles convey impressions of rhythm balanced as marvelously as is that of the heart, or the recurring symmetrical contractions of medusae. Convincing reasons are advanced for believing that the paravertebral cephalad parts of the lungs are least, and the portions at the outside about half-way between the apex and base are most, concerned with breathing. The account of what takes place when persons drown leaves little to be desired, and the description of the gross appearance of the lungs is especially to be commended. In considering the forces involved in drowning, explanations are made of why groups of air sacs dilate intermittently until they explode, why others lessen in size to even complete atelectasis, what determines distribution of the air and inhaled fluid, and which changes are primary and which secondary. The questions are stated, and the answers are set forth in deliberate, entertaining discussions interspersed with the results of investigation by others, his own experiments and illustrations and other details of the useful apparatus he devised. The studies of drowning and inhalation of dust provide convincing evidence of regional diversities in normal lungs in the movement of air, blood and lymph, as well as in respiratory capacity.

From this foundation of physiologic studies an easy and natural transition is made to studies of pulmonary disease. The author's style of separate, pointed questions prevails in the chapters on bronchopneumonia, lobar pneumonia and pulmonary tuberculosis; for each disease the same six questions serve as texts. They deal with where the inflammations start and how they spread, the causative bacteria, the rôle of secondary infections or other factors, the manner and nature of subsequent extensions of the disease, the way location in the lungs modifies what follows, and routes by which the infectious agents arrive where the disease begins. The term lobar is replaced by diffuse; broncho and lobular, by localized. The peculiarities of localized pneumonias of typhoid fever, bubonic plague, of

nurslings, the exanthematous diseases of childhood and other diseases are described. Importance of the quality of the virus is admitted, but emphasis is given to susceptibility to infection, allergy and other systemic and local conditions. Tendeloo believes that too much attention has been given to the germs. He maintains that diffuse (lobar) pneumonia is a sequel to infection somewhere else, that the inflammation in the lungs is at first deeply seated, that for its development an unusual hyperemia must first occur where the fibrin is subsequently precipitated, and that the demarcation of this hyperemia rather than anatomic barriers determines the limitation of the initial consolidation. These and other views are apparently well corroborated by the principles governing the functions of normal human lungs presented in the first half of the work. But they are also, surprising to relate, confirmed by the features of diffuse pneumonia in horses, cattle, dogs and goats. Interesting comments are made about the anatomic and clinical differences of diffuse pneumonia due to its location. Here as elsewhere the author occasionally pauses to introduce personal observations; at one period he practiced medicine for eight years.

In the masterly account of pulmonary tuberculosis, also much the longest of these studies, are assembled the salient features of contributions to this subject made by the author during almost two decades. They also served in a similar manner for the article "Pathologische Anatomie" in the "Handbuch der Tuberkulose" by Brauer, Schröder and Blumenfeld, now in its third edition.

Skilfully arranged in discussions of the six questions formulated also for nontuberculous pneumonias, this more recent review is a brief, comprehensive statement of modern problems regarding pulmonary tuberculosis. The author designates as "ideal" two of the four forms of tuberculosis he recognizes, possibly because they are more readily understood. These are both primary infections steadily progressing on the one hand to healing; on the other, to death. A third variety is also a primary infection, which becomes clinically evident, and then more or less latent with a variable subsequent course. The fourth form is ordinary phthisis with its chronicity, recurring periods of quiescence, disseminated and diffuse exudates, its cavities and systemic infection. One must read this chapter carefully to form a proper estimate of this authority's skepticism as to the rôle of allergy in tuberculosis. It is a distrustfulness comporting well with the caution and critical attitude manifest in the emphasis he gives to the inconstancy of virus potency and host susceptibility, the absence of methods of estimating such factors quantitatively and to the numerous exogenous and endogenous influences to which they are subjected. He returns again and again to these mutations and the impossibility of applying set ideas about them to the disease in any single patient. To conclude that any particular tuberculous focus in the lungs is due to a fresh exogenous reinfection, there are many requirements that must be fulfilled. It is fair to assume that the author's necropsies possess elements of finished execution and that the requirements he mentions are investigated as a routine. Restrictions of a book review prevent mention of the edifying discussion in this chapter of many other problems of pulmonary tuberculosis. The reader's interest is continually stimulated by definite suggestions pertaining to their investigation.

In the chapters on pulmonary emphysema and atypical forms of pneumonia no effort is made to answer stipulated questions. In pointing out how different parts of the lungs may be permanently, abnormally dilated by inspiratory and expiratory overdistention, Tendeloo again reverts to principles of structure and function discussed in the first part of the monograph. The differences between senile atrophy and senile emphysema of the lungs and their causes are brought out in strong relief. With other atypical pneumonias the singular and anomalous changes of the lungs in influenza are described, also the interstitial pneumonia studied by MacCallum, the aspiration pneumonia of the newly born, asthenic pneumonia and the pneumonia of old age. That occurring with psittacosis is barely mentioned probably because reports of its remarkable histologic changes appeared about the time this monograph was in press. A good index and a splendid bibliography are at the end. The references to American investigations are perhaps more numerous

than is the average in German publications. Advanced students in medicine with time for leisurely adventures in its literature will find this monograph profitable reading; for teachers, it is full of source material; for investigators of pulmonary diseases, it is a prerequisite.

Ein bewegtes Gelehrtenleben. Erinnerungen und Erlebnisse, Kämpfe und Gedanken. By Otto Lubarsch. Pp. 606. Berlin: Julius Springer, 1931.

This autobiography is of special interest to pathologists and medical men in general. The author pursued an active and varied career in pathology during the height of prewar German scientific development as well as during the war and afterward. The story of his experiences and activities under such dramatic circumstances starts in Berlin and ends in Berlin, where he was born in 1860. Though of Jewish origin, his parents were Christian in religious belief. Lubarsch has regarded himself always as a true and loyal German in the best sense, but he has been subjected more than once to bitter and unwarranted attacks on the score of a fancied antisemitism (see chapter 19). He prepared himself for medicine in the university at Leipzig and studied medicine in Jena, Berlin, Heidelberg and Strassburg, taking his doctor's degree in 1883. In Strassburg he came in contact with Jacques Loeb, the physiologist, and Eduard Kaufmann, the pathologic anatomist. After short periods of assistantship in Bern under Kronecker, the physiologist, and in Giesen under Bostroem, he served for a year and a half as assistant to Ponfick in Breslau, where he received a good training in gross pathologic anatomy. While in Breslau a row with another assistant led to a duel with pistols, in consequence of which he became, so to speak, outlawed. Unfavorable reports hindered him in obtaining a permanent appointment. He worked first with Neisser and then in Virchow's institute in Berlin. Here he first met Ostertag, the veterinary pathologist, with whom he started a few years later the publication of the Lubarsch-Ostertag *Ergebnisse der allgemeinen Pathologie und pathologischen Anatomie des Menschen und der Tiere*, which has appeared regularly ever since.

After working for some months in the zoological station at Naples, Lubarsch next became assistant to Klebs in Zurich, where he remained for about two years. Here he worked with Hanau on experimental tar cancer but without positive results. A good description is given of the condition in the laboratory and of the personality of Klebs, who at that time was so preoccupied with his tuberkulocidin and its practical use that he resigned in the fall of 1891. From Zurich Lubarsch migrated to Rostock, where he remained for several years as assistant to Thierfelder. Here he had the great luck to be married happily. This period, which otherwise was marked by disturbing experiences and controversies, ends with his removal to Posen, where he was head of the pathologic division in a newly created hygienic institute. A principal part of his duties here was to make postmortem examinations in various hospitals in Posen. He was now mainly a hospital pathologist. The complicated racial, linguistic and religious conditions in Posen in relation to scientific medicine form the subject of an entertaining account. By 1905, the situation in Posen was unbearable, and Lubarsch now spent first one year as pathologist to a hospital in Lichterfelde, where Bismarck's physician, Schweninger, was the head, and then a year in a like position in Zwickau, whence he was called to the newly created academy for practical medicine in Düsseldorf. Five years later, in 1912, he became professor of pathology in Kiel, and in 1917 he succeeded Orth in Virchow's chair in the University of Berlin, where he remained until retired on reaching the age limit. In 1917, he became editor of *Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, which he still conducts. His son was killed in the World War. The conditions during the war and after, particularly as they affected him and his work, are described graphically and without restraint. The principal episodes of the Berlin period are the struggle to maintain the right to make autopsies; the work of the committee of which he was chairman, to study and report on the so-called

Friedmann cure for tuberculosis, and the Kutisker case, in which he was accused of violating the pledge of professional secrecy because he told his class the name of the person whose organs he was demonstrating.

Separate chapters are devoted to medical education, editorial policies, political interests, travels and science in general. The account of his travels in Russia to attend medical meetings is noteworthy. At the International Medical Congress in Budapest in 1909 he again met Jacques Loeb, whom he had not seen since his days in Strassburg. The impressions of this meeting led to the following comments: "Äusserst lehrreich war es und ist es mir geblieben, dass er [Loeb] mir sagte, er würde einen Ruf an die kleinste deutsche Universität annehmen, denn die Abhängigkeit von den Geldgebern der amerikanischen Universitäten wäre drückend und auf die Dauer unerträglich. In einem solchen Rufe standen damals die königlichen und grossherzoglichen Universitäten Deutschlands; ob Loeb auch unter den jetzigen Verhältnissen, wo die Unabhängigkeit der Hochschulen durch die socialistische Herrschaft in immer zunehmendem Masse bedroht wird, den gleichen Wunsche geäussert hatte, ist mir zweifelhaft. Dass sich aber die Verhältnisse in Amerika seitdem nicht wesentblich geändert haben und die fast ausschliessliche Einstellung der meisten Amerikaner auf den Dollar deutschen und europäischen Gelehrten deutscher Vorbildung auf die Dauer das Leben dort verleidet, habe ich noch vor Kurzem erfahren, als der vor einem Jahre verstorbene hervorragende russische Histologe und Pathologe A. Maximow bei seinem letzten Aufenthalt in Berlin mir die gleichen Klagen vorbrachte wie Loeb, und wieder etwas später ein österreichischer an einem der grössten nordamerikanischen wissenschaftlichen Institute angestellter Pathologe über die ganze geistige Einstellung der amerikanischen Bevölkerung beweglich klagte." (It was and will remain to me extremely instructive that he [Loeb] told me he would accept the offer of a professorship in the smallest German university, because the dependence on the donors of the American universities was oppressive and in the long run unbearable. Such was the reputation of the royal and grand ducal universities of Germany at that time; whether Loeb would have expressed this wish under the present conditions, when the independence of the universities is more and more threatened by the socialistic regime, is doubtful. However, that the conditions in America have not changed much since then and that the almost exclusive focusing of the majority of Americans on the dollar make life in the long run unbearable for the German or European scientist of German education, I have experienced again recently, when the outstanding Russian histologist and pathologist A. Maximow, who died a year ago, at his last sojourn in Berlin, voiced the same complaints to me as Loeb, and again a little later an Austrian pathologist, who holds a position in one of the greatest North American scientific institutes, deplored the entire cultural attitude of the American people.)

Lubarsch writes clearly, seriously and earnestly. He describes persons and conditions frankly; some would say too frankly. Himself he portrays as robust, gruff and industrious, and he does not slur the fact that associates found it difficult to get along with him. Somehow he seems to fail to discuss his own researches adequately. But he does set forth his hopes and fears, his predilections and prejudices, his successes and his thwartings, and the portrait strikes one as real, as true to life.

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